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PATHOGENICITY OF TWO STRAINS OF THE TOMATO-WILT FUNGUS, FUSARIUM LYCOPERSICI SACC.[1]

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INTRODUCTION

In the studies of tomato wilt (*Fusarium lycopersici* Sacc.) conducted to date, major stress has been laid upon the development of wilt-resistant varieties of tomatoes (*15, 16, 17, 24, 33*)[3] and upon the relation of the causal organism and disease production to different environmental factors (*20, 21, 25, 27*). Reports that tomato varieties of established resistance in the areas where they were developed were succumbing to wilt in other sections of the country (*22, 26*) have indicated that the possible existence of different species, varieties, or forms of the wilt pathogene should be investigated. White (*34*) in studying this phase of the wilt problem made various tests of 24 strains[4] of *F. lycopersici* received from different sections of the country. By means of physiologic tests, he found that the various strains could be placed in one of two large groups. Members of the two groups differed markedly in virulence; however, White's tests were not made under conditions ideal for infection, and so it was deemed advisable to test the pathogenicity of a typical member of each group, on several varieties of tomato, under controlled environmental conditions. The present paper, and one dealing with the relation of excretory products of *F. lycopersici* to the production of wilt (*18*) are complementary to the investigations conducted by White (*34*).

The organism hereafter mentioned as strain A was isolated by R. P. White in 1922 at Manhattan, Kans., and designated by him strain 68. The other organism, strain B, was isolated in Louisiana by C. W. Edgerton and was reisolated at Manhattan in 1923. This culture, numbered strain 127 by White, was originally from Edgerton's culture, Louisiana 397. Both cultures were pure lined by White.

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[2] The writer wishes to express his gratitude to Dr. L. R. Jones and Dr. J. G. Dickson, of the University of Wisconsin, under whose immediate supervision the studies herein reported were conducted, for their inspirational and practical guidance. Thanks are also due Dr. R. P. White, formerly of this institution and now connected with the staff of the New Jersey Agricultural Experiment Station, and Dr. J. C. Walker, of the University of Wisconsin, for advice and critical reading of the manuscript, and to Mrs. H. H. Haymaker for assistance in much of the routine work of obtaining and tabulating data and in preparation of the results for publication.

[3] Reference is made by number (italic) to "Literature cited," p. 693.

[4] The word "strain" as used in this paper is in no way synonymous with variety, specialized race, or other terms used to designate specialization within a species. It simply refers to isolations of the fungus obtained from different localities.

CHARACTERISTICS OF THE TWO STRAINS IN CULTURE[5]

The relatively few references (*16*, *34*) to cultural characteristics of *Fusarium lycopersici* indicate that strains isolated from different sections of the country vary noticeably in such characters as color formation, spore production, nature of the mycelial growth, zonation, and others. To determine the relationship between strains A and B, comparative cultural characteristics were observed on 14 kinds of media. It was found that the two strains, while exhibiting many characteristics in common, varied distinctly as to (1) color production, (2) sporulation, and (3) nature and elevation of mycelium.

Strain A, almost without exception, produced much more color, varying from salmon pink on Richards' solution to deep, vinaceous red in plate cultures of acidified potato agar at temperatures ranging from 20° to 32° C. The mycelium of strain B varied from white to pale pink, the former predominating.

Strain A, without exception, sporulated much more profusely than strain B. Microconidia of strain A developed in abundance in practically all kinds of media, a characteristic which was not shared by strain B. Macroconidia were produced sparingly by strain A on several kinds of media; with strain B they were found but once. Varying the temperature and P_H of the media had no effect in changing this relationship between the two strains.

Strain A could be distinguished without difficulty because of its luxuriant development of aerial mycelium. The hyphae of strain B, on the contrary, were usually submerged, though occasionally powdery areas developed in Petri-dish cultures owing to a light growth of surface mycelium.

While the strain characteristics observed by White (*34*) and those here recorded agreed as to color production and nature of the mycelium, they differed markedly as to sporulation. White found that strain B produced both macro and micro conidia abundantly when grown on acidified potato agar, while strain A was practically sterile when grown under similar conditions.

Such a marked variation in results is difficult of explanation. Strain B has been shown to be extremely variable, as reisolations from diseased tomato seedlings differed from the parent strain in many ways, though they all retained the characteristic of approximate sterility as to spore production. The change in the nature of the fungus may have been due to deterioration through prolonged growth in culture (*8*), but the fact that strain B had been cultured a year less than strain A would indicate that the probable cause of the change was the accidental transfer of saltation material not evident in test-tube cultures. Brown (*6*) has shown that certain Fusaria saltate freely. He has concluded that fungi do not deteriorate gradually in culture, apparent changes of this kind being due to the accidental transfer of mycelium from a saltation sector. Edgerton and Moreland (*15*, *16*) have demonstrated also that certain strains of *Fusarium lycopersici* deteriorate only slightly when grown in culture during an extended period of time.

[5] Detailed observations may be obtained from the following: HAYMAKER, H. H., TOMATO WILT: PATHOGENICITY OF TWO STRAINS OF FUSARIUM LYCOPERSICI SACC. AND THE RELATIVE TOXICITY OF THEIR EXCRETORY PRODUCTS, 1927. [Unpublished thesis. Copy on file, Univ. Wis., Madison.]

OCCURRENCE OF SALTATION STRAINS

In an effort to obtain more rapid sporulation, strain B was exposed to a variety of conditions. When grown at 24° C. on potato-dextrose agar acidified with one drop of 25 per cent lactic acid per tube, strain B produced marginal saltation [6] sectors which differed from the remainder of the colony in color and in the nature of the mycelium. These sectors were apparently similar in nature to those occurring in other genera of fungi described by Christensen (*9*, *10*) and Leonian (*20*, *21*).

Transfers from these areas remained true to the type of the parent sectors. Petri-dish cultures, obtained from single spores, were vinaceous red in color, and there was an abundance of aerial mycelium which gave the culture a cottonlike appearance. The aerial mycelium in one isolation developed microconidia in abundance; in another, both micro and macro conidia were produced profusely. Of the several isolations made, two, designated as strains C and D, were kept in culture and later were tested for comparative pathogenicity. Throughout a series of transfers there has been no change in the characteristics of the two saltation strains.

This evidence, indicating anew the decided variability of strain B, provides additional weight for the assumption that this form was probably different from that of White's original strain 127, and that a new type had developed in the manner described by Brown (*6*).

RELATION OF TEMPERATURE TO GROWTH ON POTATO-DEXTROSE AGAR [7]

White (*34*) employed differences in growth rates on potato-dextrose agar as one of the means by which he separated 24 strains into two large groups. In view of the differences shown in cultural tests between his culture No. 127 and the writer's strain B, it was considered advisable to conduct a similar experiment to determine if strain B had changed in this respect also. Growth was determined by the increase in the size of the colonies on solid culture media, as it has been shown that this method is desirable in many respects (*5*). Standard methods used for tests of this kind were employed, and precautions were taken to make all environmental conditions as nearly uniform as possible. Two measurements were made of each colony and a large number of cultures was maintained at each temperature; so the final average was obtained from the results of either 8 or 16 determinations. The results are shown in Table 1 and are represented graphically by Figure 1. Similar results were obtained from two additional experiments in which both plain and acidified potato agar were used and in which the same methods were employed.

[6] The majority of investigators have spoken of similar sectors as mutation sectors. It has been suggested by others (*3*, *4*, *8*) that the term "mutation" should not be used to designate areas of this type in fungous cultures, inasmuch as the nature of their origin and the nuclear history involved is not understood. Consequently, in this paper, the more general term "saltation" as suggested by Stevens (*29*) will be used, thus including the possibilities that the sectors may have originated as a result of mutations, or of segregations following some type of nuclear fusion.

[7] The following formula was used: Potato, 200 gm.; dextrose, 20 gm.; agar-agar, 25 gm.; distilled water to make 1,000 c. c.

TABLE 1.—*The average diameter (in mm.) of colonies of strains A and B on potato-dextrose agar, showing the extent of radial expansion during the last five days of a seven-day period* [a]

Strain	Average diameter of colonies (mm.) at—									
	6° C.	8° C.	11° C.	15° C.	20° C.	24° C.	28° C.	31° C.	33° C.	38° C.
A	0	1	17	31	42	61	63	46	22	0
B	1	1	18	34	42	61	64	57	36	0

[a] 8 colonies were measured at each of the more critical temperatures (24°, 28°, 31°) and 4 colonies at the remaining temperatures.

It is evident that there is little difference between the growth rate of the two strains if growth be expressed by radial expansion of the colony on the medium used. The most marked difference is shown at temperatures above 28° C., at which strain B is markedly the more vigorous. This is in accord with White's findings.

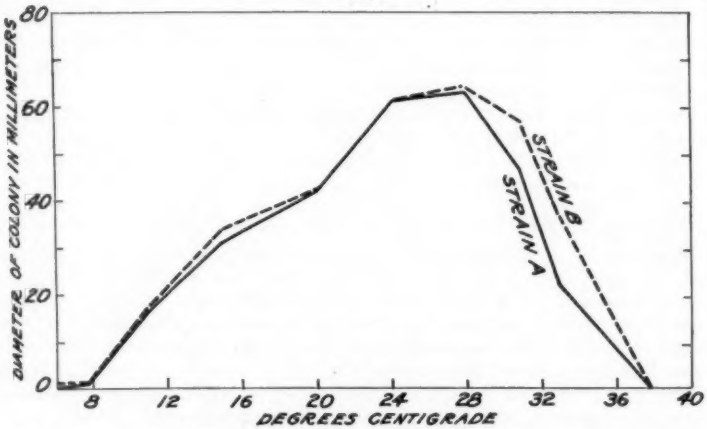

FIG. 1.—Temperature-growth curves of strains A and B of *Fusarium lycopersici* grown on potato-dextrose agar

COMPARATIVE PATHOGENICITY OF THE TWO STRAINS

Differences in virulence of strains of *Fusarium lycopersici* have been noted by various investigators (*11*, *15*, *16*). Both Clayton and Edgerton were of the opinion, however, that this variation was of no great significance. White (*34*) in testing the relative pathogenicity of 24 isolations from different parts of the United States, found decided differences in strain virulence and concluded from this evidence and other physiologic tests that the various strains could be grouped into two large subdivisions, which possibly merited distinction as varieties within the species. The possibility that *Fusarium*

lycopersici is composed of distinct races is suggested by other observations.[8]

The present experiment was designed to test the virulence, under carefully controlled environmental conditions, of a typical representative of each of the two major groups proposed by White. Seedlings were used, as it has been demonstrated that infection studies in which tomato seedlings are treated in the greenhouse produce results comparable to those obtained in field experiments with more mature plants (*15*). Seed of the standard commercial varieties was obtained from seed houses, while that of the resistant varieties was kindly furnished by F. J. Pritchard, C. W. Edgerton, and R. P. White.

METHODS

The methods involved in the preparation of the soil and the inoculum and the treatment of the host plants were essentially the same for all of the pathogenicity experiments. A soil composed of 1 part sand, 1 part manure, and 3 parts of field soil was used for all of the trials. After the soil was sterilized sufficient distilled water was added to raise the moisture to 70 per cent of its water-holding capacity, the amount required ranging from 25 to 27 per cent of the weight of air-dry soil.

Seed of different varieties of tomato was planted in 6-inch cans, which were then placed in soil temperature tanks. Soil and air temperatures of approximately 28° C. were maintained and the soil moisture was kept at a point favorable for infection (*11*, *12*). When the plants were in the second-leaf stage they were thinned, and the soil was inoculated by pouring on a heavy spore and mycelial fragment suspension obtained either from Petri-dish cultures grown on potato-dextrose agar, or from flask cultures grown on steamed barley kernels. A standardized method of producing the inoculum was employed, so that each can of plants was treated with 100 c. c. of a composite suspension obtained from the same number of 10-day-old Petri-dish cultures as there were cans to be inoculated.

The treated plants were examined each day for signs of wilting. Those that showed definite symptoms of the disease were removed, sectioned to disclose the blackened vascular system, and in case of doubt the fungus was isolated from affected tissues. At the conclusion of each experiment, usually 30 days after inoculation, the stems of the plants were sectioned at the soil line, and were examined for bundle blackening. The fungus was reisolated without difficulty from affected plants by placing a small portion of a blackened bundle on potato agar. At the conclusion of each experiment the identity of the organism was obtained after isolations were made from representative diseased plants from each can.

RELATION OF SPORE LOAD TO INFECTION

In the first experiments conducted in which the pathogenicity of the two strains was compared, strain A proved to be much the more virulent. It was recognized, however, that the relative pathogenicity

[8] F. P. McWhorter of the Virginia Truck Experiment Station, in a letter to L. R. Jones, stated that the ordinary varieties of wilt-resistant tomatoes were very unsatisfactory when planted in certain sections of Virginia where wilt was severe. He suggested that there existed distinct specialized strains or races of *Fusarium lycopersici*. His investigational work indicated that he might be able to prescribe certain varieties of tomato for use in different localities. He thus intimated that different strains of the pathogene and varieties of the host exhibited a reciprocal relationship in respect to pathogenicity and susceptibility.

could not be determined until additional information was available with regard to the quantity of inoculum necessary to produce infections characteristic of each strain.

In the earlier trials, the inoculum for each can of 25 seedlings consisted of the conidia obtained from a single Petri-dish culture, suspended in 100 c. c. of water. It was soon realized, however, that this inoculum might not be fairly representative of strain B, which sporulated much less profusely than strain A. A spore count, made with a Levy counting chamber, of the two inocula revealed the fact that microconidia from strain A were being applied at the approximate rate of 10,000,000 per cubic centimeter, while those of strain B numbered only 687,000 per cubic centimeter, a disparity in the ratio of about 15 to 1. Observational evidence indicated, however, that this difference might not be of importance, as conidia of strain B were found to have a higher percentage of viability than those of strain A. When an inoculum prepared in this manner was used to inoculate cultures in Richards' solution, the colonies from

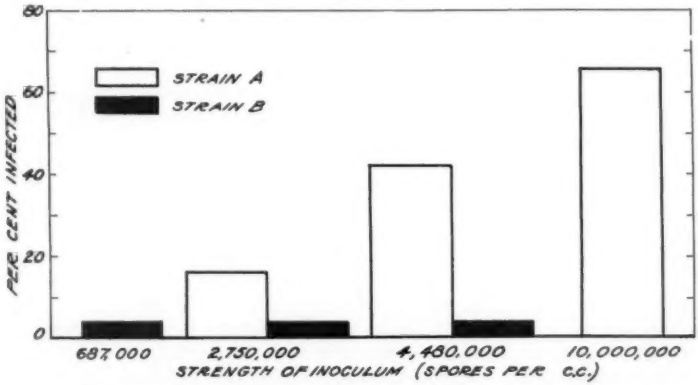

FIG. 2.—Relation of spore load to infection by strains A and B; plants grown in artificially inoculated soil at temperatures ranging from 26° to 28° C.

strain A seldom outnumbered those of strain B by more than two to one. The relative vegetative vigor of the two strains in Richards' solution was not determined by the dry-weight method, but observational evidence indicated that strain B was the more vigorous of the two.

In order to determine the importance of spore load, two experiments were conducted to test the relative virulence of the two strains; (1) when the inocula were obtained from similar amounts of Petri-dish material, and (2) when the number of microconidia was equalized. In the first experiment, seedlings of two varieties of tomatoes, Chalk's Early Jewel and New Stone planted October 28, were inoculated with representative samples of suspensions of spores of the two strains, the spores having been previously counted and the number adjusted until the inoculum of each strain contained approximately 2,750,000 microconida per cubic centimeter. The plants as usual were inoculated while in the second-leaf stage, November 17, the final records being taken 30 days later, December 17. The plants

were grown at a soil temperature of 26° to 28° C. and an air temperature ranging from 25° to 32°.

This experiment was repeated with two other varieties, Norton and a Kansas selection, 9A. The inoculum was of the same type, but was obtained from flask cultures of the two strains growing on steamed barley kernels. The spore load was equalized as before, but the number of conidia was increased, so that the concentration was nearly twice that of the previous experiment. The plants were grown under similar conditions and the data were obtained as before. The experiment was conducted between December 6 and February 3. The results of both series are shown in Table 2 and are represented graphically by Figure 2.

TABLE 2.—*Effect of spore load on infection of tomatoes of different varieties by strains A and B of Fusarium lycopersici*

Strain of F. lycopersici	Tomato variety	Percentage of plants infected by inoculum of different strengths *			
		687,000 spores per cubic centimeter	2,750,000 spores per cubic centimeter	4,480,000 spores per cubic centimeter	10,000,000 spores per cubic centimeter
A	Chalk's Early Jewel		22		77
	New Stone		10		53
	Kansas selection 9A			56	58
	Norton			28	61
	Average		16	42	65
B	Chalk's Early Jewel	6	6		
	New Stone	3	3		
	Kansas selection 9A	4		3	
	Norton	3		5	
	Average	4	4.5	4	

* In each trial the plants varied in number between 100 and 230.

The results of these two experiments show that the infecting power of strain A is diminished by reducing the number of conidia from that used in the preliminary experiments, while that of strain B is not affected by increasing the spore load. Hence it is evident that the relative pathogenicity of the two strains was tested more accurately by applying the inoculum from a single Petri-dish culture to each can, as this treatment provided more nearly optimal opportunity for infection by each strain.

RELATION OF TYPE OF INOCULUM TO PATHOGENICITY

White (*34*), in comparing the virulence of strains A and B, found the latter to be decidedly the more pathogenic of the two. This was in direct contrast to the results obtained in early experiments by the writer, in which A proved to be more virulent in every case. It was thought that the contradiction in results might have been due to the type of inoculum used, as White had inoculated the soil with a heavy concentration both of spores and mycelium obtained from flask cultures on steamed wheat kernels, while in experiments conducted by the writer, the soil was inoculated after the plants had reached the second-leaf stage, with a spore suspension poured on the soil.

In order to test the comparative efficiency of the two types of inoculum, 16 cans of soil were sterilized in the usual manner. Just prior to seeding with Kansas selection 9A, the contents of 21-day-old, 250 c. c. flask cultures of strain A and strain B were added to each of four cans and mixed thoroughly with the surface 4 inches of soil. The eight remaining cans were inoculated, four each with spore suspensions of the two strains, by pouring the suspension on the soil. The cans were kept in a greenhouse in which the temperature was held at 17° to 18° C. until the plants had reached the second-leaf stage, as it was feared that there might be a heavy loss from damping off if the young seedlings were exposed to higher temperatures with the soil so heavily infested. When the second-leaf stage was reached the cans were transferred to a chamber where the temperature varied from 25° to 30°. Observations were made in the usual manner, and the results are shown in Table 3.

TABLE 3.—*Percentage of seedlings of Kansas selection 9A which developed infection in soil inoculated at time of planting with spore suspension or with mycelial mass of strains A and B of Fusarium lycopersici. Experiment 1*

Strain	Percentage of plants dead and wilted, inoculated with—		Percentage of plants with blackened bundles, though apparently healthy, inoculated with—		Total percentage of plants affected, inoculated with—	
	Spore suspension [a]	Mycelial mass [a]	Spore suspension [a]	Mycelial mass [a]	Spore suspension [a]	Mycelial mass [a]
A	36	34	26	26	62	60
B	2	2	3	2	5	4

[a] Approximately 100 plants used in each trial.

This experiment was repeated using the same methods except that the amount of infested barley kernels applied to each can of soil was practically tripled. The spore suspension was applied as before so that each can of soil was inoculated with the conidia from one Petri-dish culture. The results are shown in Table 4.

TABLE 4.—*Percentage of seedlings of Kansas selection 9A which developed infection in soil inoculated at time of planting with spore suspension or with mycelial mass of strains A and B of Fusarium lycopersici. Experiment 2*

Strain	Percentage of plants dead and wilted, inoculated with—		Percentage of plants with blackened bundles, though apparently healthy, inoculated with—		Total percentage of plants affected, inoculated with—	
	Spore suspension [a]	Mycelial mass [b]	Spore suspension [a]	Mycelial mass [b]	Spore suspension [a]	Mycelial mass [b]
A	30	75	27	19	57	94
B	1	8	1	2	2	10

[a] Approximately 100 plants used in each trial.
[b] Approximately 150 plants used in each trial.

The results of the two experiments shown in Tables 3 and 4 indicate that the difference in pathogenicity is due to some factor or factors inherent in the two strains. Despite the fact that the inoculum of strain B was as plentiful as that of strain A, the latter was markedly the more virulent of the two. The amount of the inoculum evidently had an important bearing upon the rate of infection, as may be seen by comparing Tables 3 and 4. In the first experiment, the two types of inoculum were of equal efficiency. In the second experiment, the percentage infection induced by the spore suspension was comparable with that of the preceding trial, but that induced by the larger amount of mycelial inoculum was much higher. There is an additional possibility that the increased infection might have been partially due to the additional organic matter added to the soil when the larger amount of barley kernels was applied, as it has been shown (*16*) that the development of the disease is influenced by this factor. Whether this is due to improving the content of the soil as a medium for the development of the fungus, or to the weakening of the plants when grown in soils rich in crude fiber, has not been determined.

PATHOGENICITY OF SALTATION STRAINS

Although a great deal of progress has been made during the past few years on the isolation of new strains from saltation sectors, little has been done in testing the pathogenicity of the new isolations. Christensen (*9*) found that three of his new strains arising in this manner were less pathogenic than the cultures from which they were obtained, while two were more pathogenic. Christensen and Stakman (*10*) found that strains of *Ustilago zeae* isolated from saltation sectors varied in pathogenicity from the parent form, the new strains being less pathogenic in every case.

As the new saltation strains C and D possessed many characteristics, such as heavy sporulation and intense color production, that have been associated with high pathogenicity (*34*), their virulence was tested comparatively with that of strains A and B, standard methods of technic being employed. The experiment was concluded 51 days after the seed was planted, with results as indicated in Table 5.

TABLE 5.—*Pathogenicity of strains A and B compared with that of the strains (C and D) isolated from saltation sectors produced by strain B, to seedlings of Kansas selection 9A, grown in artificially inoculated soil held at temperatures of 25° to 30° C.; inoculum applied as seed was planted; approximately 100 plants used in each trial*

Strain	Shortest incubation period (days)	Percentage of plants dead or wilted	Percentage of plants with blackened bundles, though apparently healthy	Total percentage of plants infected
A	36	27	20	47
B	50	1	0	1
C	45	5	0	5
D	35	5	4	9

Another experiment, comparing the virulence of strains A, B, and D, was conducted along similar lines. Methods of administering the inoculum and watering the plants differed somewhat from those employed previously. The results corresponded with those obtained in the earlier experiment, the percentage infection of strains A, B, and D being 16, 3, and 9 per cent, respectively. Strain A proved less pathogenic than usual, due probably to the saturated condition of the soil (*12*).

These results show that the saltation strains, though more virulent than the parent strain B, are less pathogenic than strain A. This again demonstrates that virulence is not necessarily correlated with spore load—for the inoculum from strain D contained a far larger number of both macro and micro conidia than the inocula of the other strains tested—but is due rather to some characteristic inherent in the fungus itself.

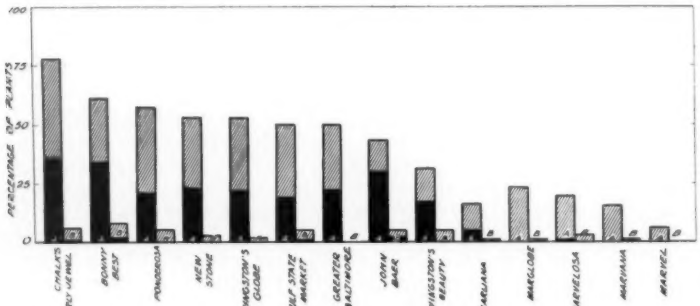

FIG. 3.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. Plants grown in artificially inoculated soil held at 28° C. Solid portion of block represents percentage of plants dead or wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy. Series 1

PATHOGENICITY OF STRAINS A AND B TO SEEDLINGS OF DIFFERENT TOMATO VARIETIES UNDER CONDITIONS FAVORABLE FOR INFECTION

Breeding for disease resistance is rendered more complex by the existence of specialized races of the pathogene which vary in their pathogenicity toward different hosts. This is especially true when additional biologic forms exist that parasitize the varieties of the host first found to be resistant, as is true in the case of the two wheat rusts (*23*, *28*), bean anthracnose (*1*, *2*, *7*, *19*), and corn smut (*10*). To determine if a similar relationship existed between strains of *Fusarium lycopersici*, it was deemed advisable to test the virulence of strains A and B on a representative group of field and greenhouse varieties of tomatoes; also on those that have proved most successful in the several sections of the country in resisting wilt.

VARIETAL TEST NO. 1

In this experiment 10 commercial and 4 of the resistant varieties developed by Pritchard were tested. The soil, which had a P_H of 7.7, was prepared and inoculated in the usual manner. The results shown in Table 6 are represented graphically in Figure 3. Variations in the pathogenicity of the two strains to variety Greater Baltimore are illustrated in Figure 5, C, D.

TABLE 6.—*Pathogenicity of strains A and B of Fusarium lycopersici to different varieties of tomato grown in artificially inoculated soil held at 27° to 28° C., seed planted August 27, plants inoculated September 14, experiment concluded October 13, 1926, approximately 100 plants used in each trial*

Variety of tomato	Shortest incubation period (days)		Percentage of plants dead or wilted		Percentage of plants with blackened bundles, though apparently healthy		Total percentage of plants infected	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Chalk's Early Jewel	15	28	36	1	41	5	77	6
Bonny Best	19	28	34	2	28	6	62	8
Ponderosa	22	------	21	0	37	5	58	5
New Stone	20	------	23	0	30	3	53	3
Livingston's Globe	22	------	22	0	32	2	54	2
Gulf State Market	22	29	19	1	32	4	50	5
Greater Baltimore	22	------	22	0	28	0	51	0
John Baer	22	27	30	2	12	3	42	5
Livingston's Beauty	21	------	18	0	13	5	31	5
Earliana	22	------	5	0	11	1	16	1
Marglobe	------	------	0	0	23	1	23	1
Marvelosa	22	------	1	0	19	3	20	3
Marvana	------	------	0	0	16	1	16	1
Marvel	------	------	0	0	6	0	6	0

VARIETAL TEST NO. 2

The soil, though prepared as before, had a slightly higher water-holding capacity. The P_H of the soil, which measured 7.6, was essentially the same as that in the other series. Commercial field varieties, English forcing varieties, and others selected for resistance by Pritchard, by Edgerton and Moreland, and by White were tested. The results are shown in Table 7 and represented graphically by Figure 4 and by illustrations in Figure 5, A, B.

TABLE 7.—*Pathogenicity of strains A and B of Fusarium lycopersici to different varieties of tomato grown in artificially inoculated soil held at 27° to 28°; seed planted September 29, plants inoculated October 24, experiment concluded November 22, 1926; approximately 100 plants used in each trial*

Variety of tomato	Shortest incubation period (days)		Percentage of plants dead		Percentage of plants wilted		Percentage of plants with blackened bundles		Total percentage of plants infected	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Carter's Sunrise	25	31	31	2	11	1	16	4	58	7
Sterling Castle	20	35	30	3	3	0	16	4	49	7
Kanora 6	25	35	24	3	8	0	15	1	47	4
Norton	25	30	21	3	12	0	12	0	45	3
Kanora 11	26	29	30	7	6	3	7	3	45	13
Monumental	25	29	27	7	8	1	10	3	44	11
Ailsa Craig	33	------	19	2	6	0	7	10	32	12
Norduke 3	25	29	13	3	7	1	10	1	30	5
Kansas selection 240–1–1	27	35	19	0	3	1	7	0	29	1
Avon	------	------	11	5	5	2	11	2	27	9
Bide's Recruit	33	35	15	0	3	1	6	2	24	3
Louisiana Pink	27	35	5	0	4	1	15	1	24	2
Kansas selection 220–1–2	25	35	7	1	4	1	11	1	22	3
Kansas selection 205–3	25	------	7	0	9	0	5	1	21	1
Louisiana Red	32	------	4	0	3	0	12	0	19	0
Kansas selection 205–1–1	30	35	5	1	3	0	4	2	12	3

These two varietal tests demonstrated several significant facts. (1) None of the varieties tested is entirely immune to the attack of the more pathogenic strain of the wilt fungus. (2) Varieties that have proved highly resistant under field conditions may be quite susceptible when conditions for infection are more favorable. This was shown in an especially marked manner in the trials of Norton and Kanora 11. With few exceptions, Norton has been reported resistant under an extremely wide range of conditions. Kanora 11, when tested in comparison with other varieties in Kansas, has proved very resistant under field conditions (*33*). (3) At least part of the resistance shown by certain varieties may be attributed to the plant's ability to produce a partial crop in spite of the presence of the pathogene in the host tissues. This merely adds some additional evidence to similar conclusions advanced by Edgerton and Moreland (*16*) and recognized by those who have studied tomato wilt critically. (4) There is a marked difference in the virulence of the two strains. In all of the varieties studied, strain B at no time produced higher than 12 per cent infection and in several cases was unable to cause any infection whatsoever. (5) There was no evidence of reciprocal

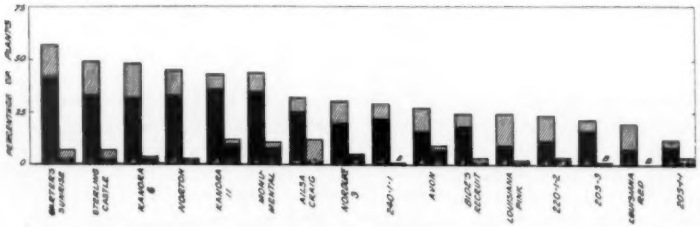

FIG. 4.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. Plants grown in artificially inoculated soil held at 28° C. Solid portion of block represents percentage of plants dead and wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy. Series 2

resistance and susceptibility by different varieties to the two strains of the fungus. (6) Results obtained with some varieties did not agree with reports of the disease in the field. Livingston's Globe has been generally reported to be one of the most resistant of the commercial varieties, while the ability of Earliana to produce fair yields has been attributed to freedom from disease through early maturity (*24*). Under conditions favorable for infection, Livingston's Globe proved to be highly susceptible to strain A, while the evidence indicated that Earliana possesses factors other than early maturity which are of value in warding off the attack of the parasite. Greater Baltimore, regarded as one of the most susceptible varieties, while subject to the attack of strain A, seemed immune to strain B.

VARIETAL TEST NO. 3.

Because of the limited time left for the use of the temperature tanks, it was not possible to repeat the combined pathogenicity-varietal test with varieties other than those that had shown some characteristic that merited special consideration. Thus, Livingston's Globe, Earliana, and Greater Baltimore were tested a second time to determine if the results obtained before could be duplicated.

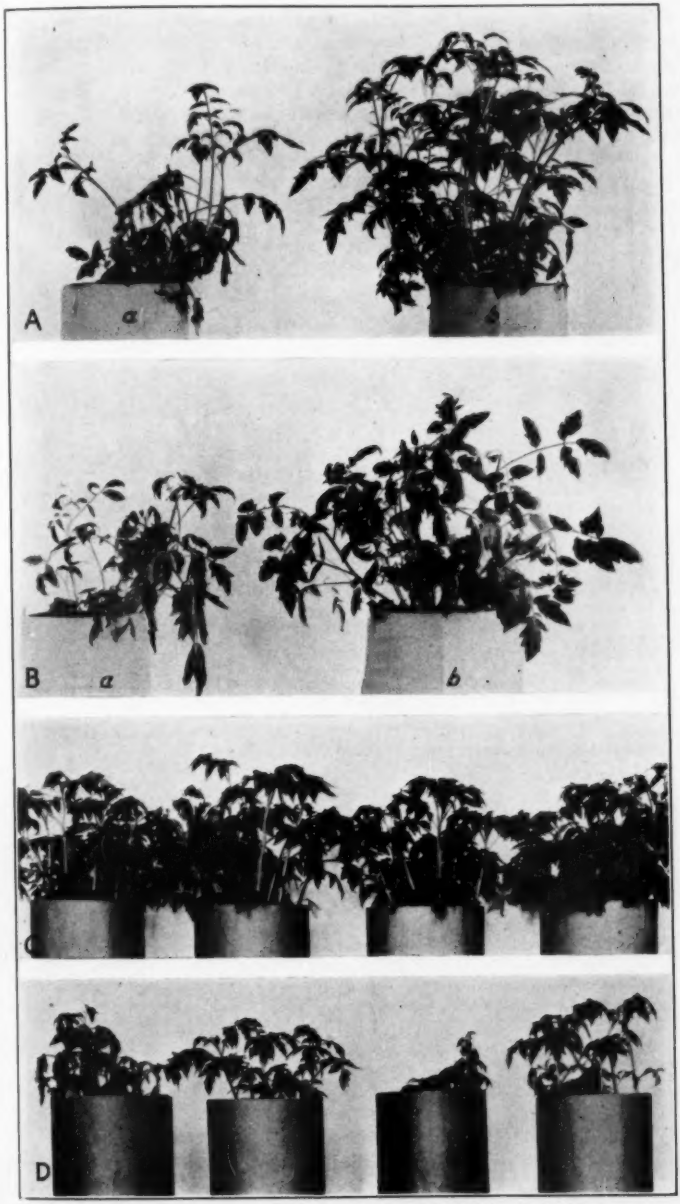

FIG. 5.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. A, to Norton: *a*, strain A; *b*, strain B. B, to Kanora 11: *a*, strain A; *b*, strain B. C, virulence of strain B to Greater Baltimore. D, virulence of strain A to Greater Baltimore

The only departure from the methods used in the two former experiments was that the seed was allowed to germinate in a cool greenhouse and the young seedlings were held at temperatures ranging from 18° to 22° C. for three weeks before they were placed in the temperature tanks at 28°. The results of the experiment are shown in Table 8 and are represented graphically in Figure 6.

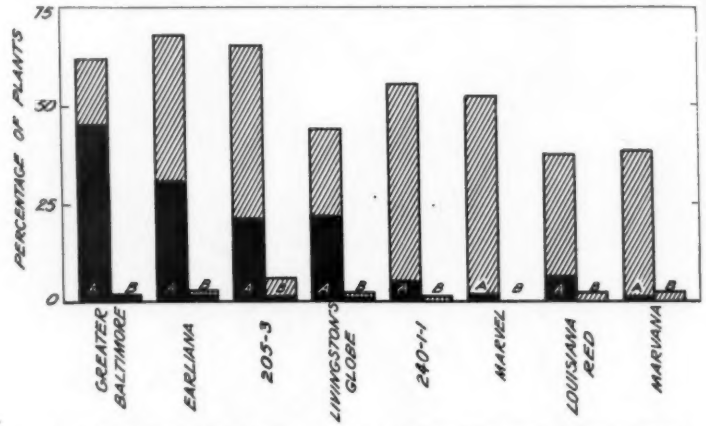

FIG. 6.—Relative pathogenicity of strains A and B of *Fusarium lycopersici* to different varieties of tomato. Plants grown in artificially inoculated soil held at 28° C. Solid portion of block represents percentage of plants dead and wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy. Series 3

TABLE 8.—*Pathogenicity of strains A and B of Fusarium lycopersici to different varieties of tomato grown in artificially inoculated soil held at 28° C.; seed planted February 11, plants inoculated March 11; experiment concluded April 7, 1927: approximately 100 plants used in each trial*

Variety of tomato	Shortest incubation period (days)		Percentage of plants dead		Percentage of plants wilted		Percentage of plants with blackened bundles, though apparently healthy		Total percentage of plants infected	
	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Greater Baltimore	14	23	34	1	11	1	17	0	62	2
Earliana	19	27	26	0	5	2	38	1	69	3
Kansas selection 205–3	19	24	17	1	4	0	44	4	65	5
Livingston's Globe	19	27	18	0	4	1	23	1	45	3
Kansas selection 240–1–1	26	.....	3	0	2	0	48	1	53	1
Marvel	27	.....	0	0	2	0	50	0	52	0
Louisiana Red	22	.....	4	0	2	0	31	2	37	2
Marvana	19	.....	1	0	0	0	38	2	39	2

The results of this experiment were noteworthy in at least three respects. (1) The apparent constancy in the virulence of strain B and the greater pathogenicity of strain A were again demonstrated. (2) The ability of certain varieties to thrive for a time at least even

though the plants were heavily invaded, as shown by the number having blackened bundles, was demonstrated much more conclusively than in the earlier experiments. This was especially marked in the Kansas selection 240–1–1, in Marvana, and in Marvel, as the data show that the pathogene invaded a high percentage of the plants of these varieties without having displayed external indications of its presence. Plants of the variety Marvel, although infected to over 50 per cent in the strain A series, evidenced no more signs of a diseased condition externally than those treated with strain B in which

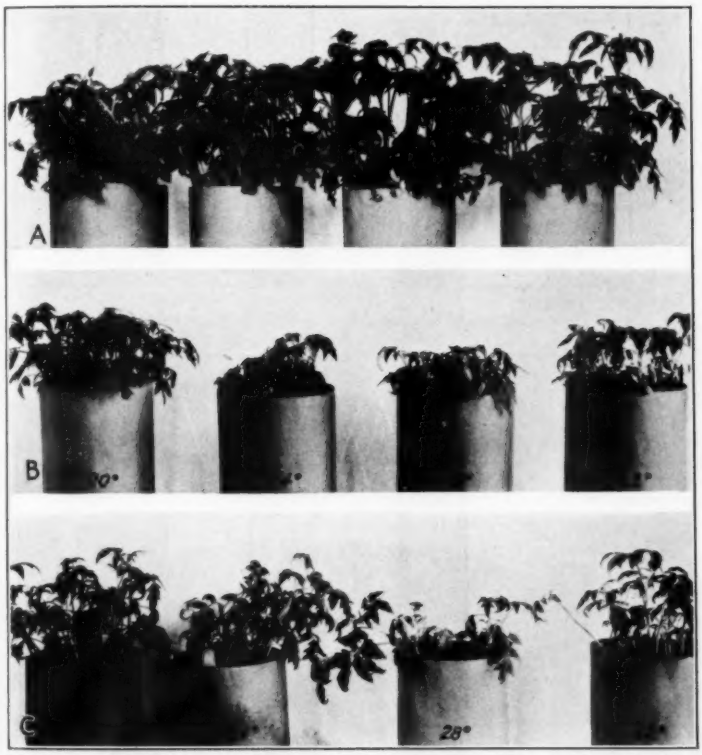

FIG. 7.—A.—Tolerance shown by Marvel seedlings to *Fusarium lycopersici*; 52 per cent of seedlings shown had blackened vascular bundles. B and C.—Seedlings of Kansas selection 9A, showing the relation of soil temperature to production of tomato wilt by a strain of *Fusarium lycopersici*; temperatures (°C.) as indicated

the infection was zero. (Fig. 7, A.) (3) With a single exception strain A proved to be more pathogenic than in the previous experiments. This might have been due to the fact that the strain had become more virulent while in culture, a development that would scarcely be expected since strain A had been stable and not at all inclined to saltate; or it might have been that the exposure of the plants to low temperatures prior to their inoculation had predisposed them to infection by the wilt fungus. The latter explanation would conform with results obtained in the study of certain other Fusarium

diseases and would seem to be the more likely (*14*, *30*). This possibility is of such significance, from a practical as well as a scientific viewpoint, that it merits further consideration.

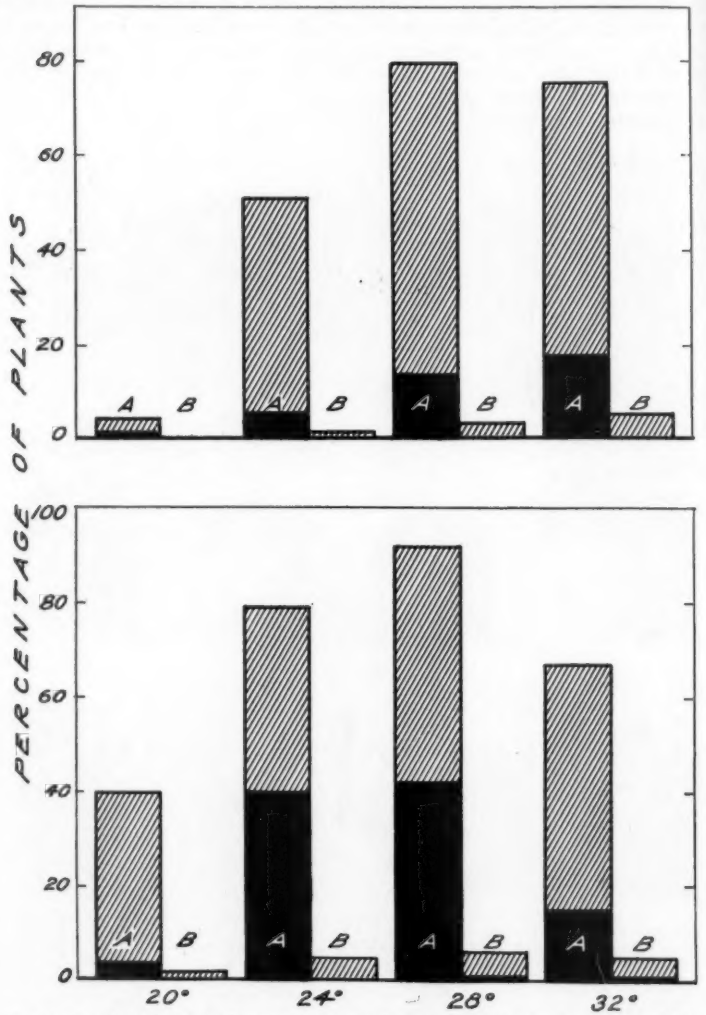

FIG. 8.—Seedlings of varieties Norton (upper) and Kansas selection 9A (lower), showing relation of soil temperature to wilt production. Plants grown in artificially inoculated soil. Solid portion of block represents percentage of plants dead and wilted; lined portion represents percentage of plants with blackened bundles, though apparently healthy

RELATION OF SOIL TEMPERATURE TO PATHOGENICITY

Considerable evidence has accumulated during the past few years demonstrating the close relationship existing between soil temperature and the inception of certain diseases. In most of these experi-

ments, the relation of temperature to the development of the fungus on some solid culture medium, usually potato agar, has been determined and attempts have been made to correlate this with the relation of temperature to the development of the disease. Such relationships have coincided closely in the case of some of the diseases caused by vascular-invading Fusaria (*31, 11, 16*). The correlation is not so close in flax wilt (*32*), while in the case of corn root rot, in which the cortex is invaded, temperatures favoring the growth of the organism inhibit the development of the disease (*13*).

Clayton's investigations on the relation of temperature to infection by *Fusarium lycopersici* were limited to one strain of the fungus, to one variety of the tomato, and to the use of semimature plants only. His work was repeated in part by the writer for the following reasons: (1) To learn if infected seedlings react to different soil temperatures in a manner similar to that of older plants; (2) to ascertain if the results would be affected by the use of different host varieties grown directly from seed; (3) to learn more of the relation of temperature to resistance, and (4) to determine if the two strains of the pathogene show variations in susceptibility at different temperatures. White (*34*) found that strain B was much more virulent than A. His results were obtained with soil held at a lower temperature than that used in the writer's experiments. Because of this it was thought that his results, which were the reverse of those obtained in the present investigations, might have been due to the fact that strain B was favored by lower temperatures. If such were the case it would also have a practical bearing on greenhouse culture.

TABLE 9.—*Percentage of plants of varieties Norton and Kansas selection 9A which showed infection when grown in soil of different temperatures inoculated with strains A and B of Fusarium lycopersici; seed planted January 10, plants inoculated February 6, experiment concluded March 9, 1927; approximately 100 plants used in each trial*

Variety of tomato	Average soil temperature	Shortest incubation period (days)		Percentage of plants dead		Percentage of plants wilted		Percentage of plants with blackened bundles, though apparently healthy		Total percentage of plants infected [a]	
		Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
	°C.										
Norton	20	26	.....	1	0	0	0	3	0	4	0
	24	24	.....	2	0	3	0	45	1	50	1
	27.5	19	.....	11	0	2	0	66	3	79	3
	32	16	.....	8	0	9	0	58	5	75	5
Kansas selection 9A	20	25	.....	1	0	3	0	35	2	39	2
	24.5	19	.....	29	0	11	0	39	5	79	5
	28	17	25	37	1	5	0	50	5	92	6
	32	18	28	10	0	5	1	52	3	67	4

[a] A total of 476 plants, used as controls, showed no infection.

In order to determine the optimum temperature for the production of the disease on seedlings by each of the two strains, two experiments were conducted. Norton and Kansas selection 9A were selected for these trials in order that a resistant and a susceptible variety might be tested. Seed of these varieties from close pollinated plants was used. As it had been shown by Clayton (*11*) that his Indiana

strain of *Fusarium lycopersici* was pathogenic throughout the range 20° to 32° C., the temperatures were maintained at approximately 20, 24, 28, and 32 degrees. The soil was prepared and the inoculations were carried out as in the foregoing experiments. The results shown in Table 9 are represented graphically in Figure 8 and are illustrated by Figure 7, B and C.

The results of the studies with seedlings were in agreement with those previously obtained by Clayton (*11*) with more mature plants grown from cuttings. This was especially true of the strain A series in which infection was sufficiently heavy to give results of experimental value. Although the higher temperatures favored the production of wilt by strain B, the percentage of infection was so small that the variation in disease production at the different temperatures was not marked.

Temperatures favoring infection coincide fairly accurately with those promoting the radial expansion of the fungus on potato agar. Twenty-eight degrees was the optimum for disease production and for the radial extension of the fungus in culture. The fungus, on potato agar, grew almost as rapidly at 24° C., and the results with Kansas selection 9A show that it is possible for the disease to develop readily at this temperature. In the variety Norton, although there was a high percentage of bundle blackening at 24°, there was little wilt, temperatures above 28° C. apparently being necessary to the early development of the disease in a severe form. Results practically duplicating these were obtained in another experiment conducted in a similar manner. These data indicate that some varieties may be especially valuable in certain localities and during seasons in which soil temperature falls somewhat below 28°. Apparently some varieties may succumb to the disease at relatively low temperatures, while others retain their disease resistance.

DISCUSSION

If the differences in the two strains studied are characteristic of isolations from different parts of the country, *Fusarium lycopersici* is an extremely variable fungus. This variability may possibly be explained by the facility with which, when the fungus is subjected to favorable conditions, its nature may be changed by saltations. The possibility of the origin and dispersal of strains aggressively parasitic upon varieties now considered resistant, should not be underestimated. There is a remote possibility that variations in environmental conditions may be of importance, not alone in their effect upon the association of host and parasite, but also in inducing the fungus to change its inherent nature through general saltations.

Although the two strains exhibited marked differences in virulence, there was no case in which varieties which were resistant to the more virulent form of the fungus succumbed to the other strain. Hence the problem of breeding for resistance would not be so complicated as in the case of diseases like the cereal rusts and bean anthracnose. In addition, the strains of the tomato wilt fungus lack the stability characteristic of forms of the cereal rust fungi. Consequently, from the evidence obtained in these experiments, there would seem to be no value in attempting to establish varieties or forms of *Fusarium lycopersici*.

SUMMARY

Comparative physiologic tests were made with two strains of *Fusarium lycopersici* Sacc., obtained from widely separated localities. The two strains were typical representatives of White's (*34*) dissimilar groups.

The two strains showed markedly different characteristics in culture. Strain A by several criteria proved to be very constant; strain B proved extremely variable, producing many saltations which differed from the parent form in appearance and in pathogenicity.

Without exception, strain A proved to be more pathogenic than strain B. The difference in pathogenicity could not be altered by regulating the soil temperature, increasing the spore concentration in the inoculum, or by using different types of inoculum.

A soil temperature of 28° C. proved the optimum for the production of wilt in seedlings of two varieties. A similar temperature proved to be the optimum for the lateral spread of colonies of the two strains on potato-dextrose agar.

The lack of stability displayed by different strains and the fact that variations in strain virulence were uniform on all host varieties tested, make it inadvisable, from the evidence obtained in these expriments, to establish varieties or forms of *Fusarium lycopersici*.

Resistance in certain varieties is apparently correlated with two factors. (1) The temperature range over which certain varieties are susceptible may be broader or may be different from that over which the more resistant varieties are subject to infection. This was indicated by preliminary trials in which Norton was more susceptible than Kansas selection 9A at 31° C., but was decidedly more resistant at 24° C. The divergence in results with resistant varieties obtained by workers in different localities may have been due to this characteristic. (2) Resistant varieties apparently possess certain physiological characteristics that enable them to tolerate the final attack of the pathogenein spite of its invasion of the host tissues. This was shown strikingly in the case of Marvel, which evidenced but 2 per cent wilting in spite of 52 per cent infection.

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RELATION OF TOXIC EXCRETORY PRODUCTS FROM TWO STRAINS OF FUSARIUM LYCOPERSICI SACC. TO TOMATO WILT[1]

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REVIEW OF LITERATURE

Of the several theories which have been advanced to explain how wilting is produced by vascular-invading pathogenes, White (*26*)[3] and Rosen (*21*) have mentioned five, viz, (1) the parasitism theory of Atkinson (*1*), (2) the bundle-plugging theory, first elaborated by Stewart (*24*) in bacterial diseases, and by Smith (*23*) in fungous diseases and developed further (*4*, *13*, *22*) to include obstruction of vessels by products of the pathogene; (3) the rootlet-destruction theory first advanced by Orton (*16*); (4) the embolism theory of Tochinai (*25*); and (5) the toxin theory, advanced by Hutchinson (*13*) and further elaborated by Goss (*8*), Brandes (*6*), and many others during the past decade. Neither White nor Rosen mentioned the theory propounded by Van der Lek (*15*), that the actual wilting was due to the invasion of the leaf parenchyma by the organism. Van der Lek arrived at this conclusion after proving that the cucumber leaf did not wilt despite the invasion of the petiole by the mycelium of Verticillium until the hyphae had progressed to the lamina.

The greater part of the work conducted relative to the toxin theory has dealt with the nature of the toxic principle, and important results have been obtained. Nearly all of the investigations (*3*, *4*, *5*, *8*, *9*, *19*, *21*, *26*, *27*, and that by Tims[4]) dealing with metabolic products in liquid cultures of fungi have demonstrated that the toxic materials are for the most part thermostable. The sole evidence to the contrary has been furnished by later work of Goss (*10*). Specific principles, however, isolated from the filtrate by precipitation with alcohol, primarily enzymic in nature, have been partially (*4*) or wholly (*26*) inactivated by boiling.

Conflicting evidence has been obtained concerning the volatility of the toxic principle. Barnum (*3*), Fahmy (*9*), and Young and Bennett (*27*) concluded that it was nonvolatile. White (*26*) and Rosen (*21*) found initial fractions of distillation toxic to tomato and cotton plants. Lathrop (*14*) found that *Fusarium cubense* produced a volatile aldehyde which he thought might possibly be toxic.

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[3] Reference is made by number (italic) to "Literature cited," p. 718.

[4] TIMS, E. C. STUDIES ON THE FUSARIUM DISEASE OF CABBAGE. 1924. [Unpublished manuscript. Copy on file, Library, Univ. Wis., Madison.]

Likewise, there is a difference of opinion regarding the relation of reaction of the filtrate to toxicity. Young and Bennett (*27*) and Tochinai (*25*) concluded that an increase in P_H was correlated with increased toxicity. Apparently they did not take into consideration the increased toxicity due to aging (*5*). On the other hand, Fahmy (*9*) and Bisby (*5*), while noting that the medium in which the fungus was growing became more alkaline, thought this change of little importance as a direct factor in causing affected plants to wilt.

Some progress has been made in separating from the filtrate the component materials which are capable of producing effects similar to wilt. Picado (*19*), Bewley (*4*), and White (*26*), working with various fungi, isolated exo- and endo-enzymes which were capable of causing symptoms resembling the fungous wilt. Picado also isolated a thermostable substance which was much more toxic than the enzymic material. White, by separating the components of the filtrate by dialysis, obtained thermostable materials, crystalloidal in nature, that were somewhat toxic to tomato plants. He as well as Bewley found that the filtrate would not diffuse through the membranes of an uninjured root system and cause wilting. This observation suggested that the toxic principle was colloidal in nature. Barnum (*3*) and Rosen (*21*) obtained results contrary to these.

That noncolloidal materials might have some bearing upon wilting is suggested by the results of Haskell (*11*), Peltier (*18*), and White (*26*), who showed that symptoms similar to those produced by treatment with fungal extracts developed when plants were treated with dilute solutions of various organic acids. Fahmy (*9*) concluded that ammonia and oxalates, while present in the filtrate of *Fusarium solani*, occurred in quantities insufficient to cause wilting. Rosen (*21*) obtained evidence indicating that an inorganic salt, a nitrite, might be one of the toxic ingredients causing wilt of cotton.

Little has been done to date in attempting to correlate the action of the toxic materials from liquid cultures with the corresponding effect resulting from the action of the parasitic fungus on the host. It has been shown repeatedly (*3,5,6,9,19,26,27*, and by Tims[5]) that metabolic products from fungi lack the specificity displayed by the organisms from which they were derived, the filtrate from saprophytic forms having proved as toxic apparently as that from vascular Fusaria. On the other hand, Rosen (*21*) found that the toxicity of the filtrate of different fungi to cotton seedlings was in direct correlation with the taxonomic relationship of the organisms themselves. Also, White (*26*) showed that a variety of tomato resistant to *Fusarium lycopersici* showed greater resistance to the action of the filtrate of that fungus than did a susceptible variety. Tims[5] obtained some evidence indicating that *F. conglutinans* grown at a relatively low temperature produced materials of greater toxicity than when grown at temperatures approaching the optimum for the vegetative vigor of the fungus.

While investigations have dealt for the most part with the relation of products of the pathogene to wilting, the evidence presented by Haskell (*11*) and Overton (*17*) suggests also that decomposition products from dead host cells may play some part in the production of wilt.

[5] TIMS, E. C. Op. cit.

DEFINITION OF PROBLEM

The experiments on varietal response to two strains[6] of *Fusarium lycopersici* (*12*) indicated that their pathogenicity and the resistance of certain varieties might be explained to better advantage if the factor or factors responsible for wilting were known. Evident resistance of varieties like Marvel, which showed practically no sign of the disease despite the invasion of its tissues by the fungus, suggested that wilting was not caused primarily by bundle plugging. Evidence pointed to the fact that the products of the fungus must have some harmful effect upon the host tissues. Three leads were opened by the earlier experiments (*12*) which should be correlated with studies of the excretory products of the wilt fungus. Consequently, experiments were planned that would furnish some evidence on the following questions: (1) Are the excretory products of the two strains of equal toxicity? (2) Do different varieties of the host show variations in susceptibility toward the toxic products of the causal fungus that are comparable to their reaction toward the pathogene itself? (3) Is there any correlation between the effect of different temperatures upon the pathogenicity of the fungus and the toxicity of its metabolic products? If there is a positive correlation between pathogenicity of the organism and toxicity of its products, as expressed by these three relationships, some additional evidence might be gained incriminating toxic excretory products as the cause of wilting.

EXPERIMENTAL METHODS

The methods of procuring the various toxic materials from liquid cultures of *Fusarium lycopersici* used in conducting the experiments were as given below.

The fungus was grown in liter flasks in 250 c. c. of Richard's solution[7] modified slightly by substituting dextrose for sucrose. The growth period varied in the different experiments, ranging usually from three to five weeks. At the close of this period the liquid portion of the culture was filtered through a double thickness of Carl, Schleicher, and Schüll's No. 590 filter paper in a Büchner funnel.[8] In those tests in which it was thought that wilting might not take place for several days the original filtrate was refiltered in a similar manner.

A double thickness of parchment paper was used as the selective membrane in those experiments in which the colloidal and crystalloidal materials were separated by dialysis. The material to be treated was dialyzed with distilled water for a period of 28 hours.

In an effort to obtain extracellular enzymes the filtrate from the cultures in Richard's solution was treated with an equal amount of 95 per cent alcohol. After a period of 24 hours most of the supernatant liquid was decanted or siphoned off, the precipitate was removed from the remainder by filtration, was rewashed with 95 per cent alcohol and again with ether, and was allowed to dry at laboratory temperature. It was then scraped off the filter paper and finally redissolved in an amount of distilled water equaling that of the original filtrate.

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[6] The term "strains" as used in this paper refers simply to the two isolations of *Fusarium lycopersici* reported upon previously (*12*).

[7] The following formula was used: KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 20 mgm.; dextrose, 50 gm.; and water, 1,000 c. c.

[8] Results of experiments proved that filtration through the finer-grained porcelain filters was unnecessary, as tomato seedlings placed in heavy spore suspensions of *Fusarium lycopersici* showed no signs of wilting after 48 hours, by which time seedlings placed in the toxic solutions had wilted completely. (Fig. 1, A. B.)

The plants to be treated were hardened at 18° C. until the second or third leaf stage was reached and were then transplanted to pots and allowed to grow for three or four weeks at temperatures of 25°–32° C. By treating large numbers of seedlings in this way, stocky, vigorous plants of uniform size were obtained. These were cut below water before being placed in the different solutions.

Young plants thus treated usually wilt in a typical manner, the wilting process occurring in a definite sequence of stages. In order to indicate the relative toxicity of the products of the two strains, certain numerical values were assigned to the various stages of the wilting process from which an artificial "index of toxicity" could be recorded. The first definite sign of an abnormal condition is expressed by a flaccid condition of the plant. This is followed first by a drooping of the stem, and then after a rather protracted interval by a loss of turgor in the leaflet laminae. This condition is succeeded by the collapse of the leaflets. That this stage, rather than the initial drooping of the stem, more closely parallels the true wilt is suggested by the fact that the plant is seldom able to recover from this condition. After the leaflets collapse they first become water-soaked and finally dry and brittle. The only exception to this sequence of stages in the wilting process is found when exceptionally stocky, vigorous plants are placed in extracts of low toxicity, in which case the initial stem drooping is often replaced by a marginal wilting and an inward rolling of the laminae of the leaflets.

TOXICITY OF THE EXCRETORY PRODUCTS FROM STRAINS A AND B OF FUSARIUM LYCOPERSICI TO TOMATO PLANTS OF DIFFERENT VARIETIES IN DIFFERENT STAGES OF MATURITY

TOXICITY OF EXCRETORY PRODUCTS TO PLANTS IN SEEDLING STAGE

The results of the varietal tests (*12*) demonstrated that there was a distinct difference in pathogenicity between strains A and B and also that the several varieties of tomato tested varied markedly in susceptibility. In order to determine the relation that might exist between the virulence of the fungus and the toxicity of its products, an experiment was conducted in which the same varieties were treated with different products of the fungus.

In the first series 14 varieties were treated with three different types of material—(1) the filtrate, which contained all of the toxic principles; (2) a redissolved alcoholic precipitate, which preliminary tests showed to be primarily colloidal in nature; and (3) the diffusate of the dialyzed filtrate, which consisted of crystalloidal materials. Each flask containing 250 c. c. of Richards' solution was inoculated with 10 c. c. of a heavy spore suspension. One-half of the cultures were inoculated with strain A, the remainder with strain B. The cultures were 3 weeks old when the mycelial mat was removed by filtration. The colloidal and crystalloidal materials were obtained in the usual manner. Plants of the different varieties were grown in sterile soil from seed planted 40 days earlier. Each plant was pulled up by the roots and the stem was cut under water before it was placed in the solution. Sterile distilled water and Richards' solution were used for controls.

The first evidence of drooping was noticed in the plants subjected to Richards' solution. They showed a slight flagging within an hour after being placed in the extract. That this flagging was caused by

the concentration of the medium was indicated by the fact that the plants regained their turgidity some hours later, as the osmotic relation between the solution and the cell sap became more nearly balanced.

In this experiment the colloidal materials in the alcoholic precipitate were practically innocuous; so the data were not included with that of the remaining treatments. This lack of toxicity was probably due to the comparatively short length of time during which the fungus had been growing, and to the fact that all of the colloidal materials were not recovered in the precipitate. The results obtained with the filtrate and the crystalloidal materials of the diffusate are summarized in Table 1.

TABLE 1.—*Toxicity of the excretory products of strains A and B, obtained from the filtrate of a 3-weeks-old culture grown on modified Richards' solution, to seedlings of 14 varieties of tomato*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Nature of variety as demonstrated by seedling tests in greenhouse	Variety of tomato	Effect of filtrate						Effect of crystalloidal materials						Index of toxicity; sum of observations	
		Hours required to produce first stem drooping		Index of toxicity after 10 hours		Index of toxicity obtained from the sum of three observations, taken after 4, 10, and 22 hours		Hours required to produce complete brittling of leaves		Index of toxicity after 30 hours		Index of toxicity obtained from the sum of three observations			
		Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Susceptible	Chalk's Early Jewel	3	5	6	5	20	12	56	62	5	1	18	12	38	24
	Bonny Best	10	10	6	6	20	18	62	62	6	6	18	18	38	36
	Ponderosa	10	10	5	5	18	13	56	56	7	6	25	19	43	32
	New Stone	10	22	6	0	14	8	62	62	5	5	17	18	31	26
	Livingston's Globe	10	10	5	5	13	13	56	62	6	6	19	18	32	31
	Gulf State Market	10	10	5	5	11	11	62	62	7	7	20	20	31	31
	Greater Baltimore	9	10	6	5	19	18	48	56	7	6	27	19	46	37
	John Baer	3	3	6	6	20	20	48	48	7	7	25	24	45	44
	Earliana	10	22	6	0	14	8	48	62	6	6	26	19	40	27
Moderately resistant	Livingston's Beauty	22	22	5	5	13	11	62	62	6	6	18	18	31	29
Resistant	Marglobe	31½	10	6	5	19	13	62	62	5	1	18	11	37	24
	Marvelosa	10	10	5	5	11	11	62	62	5	5	18	17	29	28
	Marvana	22	22	0	0	8	6	56	56	5	5	18	18	26	24
	Marvel	22	22	0	0	6	6	56	62	5	5	19	16	25	22
Total				67	52	206	168			82	72	286	247	492	415

In the second trial the 16 varieties tested in the second varietal-pathogenicity series (*12*) were exposed to the three types of toxic materials. The methods resembled those used in the first trial with two notable exceptions—(1) the strains of the fungus were allowed to grow in culture for five weeks instead of three and (2) the alcoholic precipitate was redissolved in an amount of water equaling but one-half that of the filtrate from which it was obtained. This provided a more concentrated solution, which in many cases caused initial stem drooping more rapidly than did the filtrate. Plants in the latter solution, however, wilted permanently more quickly in spite of its lighter concentration. The results are shown in Table 2.

TABLE 2.—*Toxicity of different excretory products from 5-weeks-old cultures of strains A and B grown on Richards' solution to the seedlings of 16 varieties of tomato*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Nature of variety as demonstrated by seedling tests in greenhouse	Variety of tomato	Effect of filtrate						Effect of products (mainly colloidal) in the alcoholic precipitate						Effect of crystalloidal products						Index of toxicity; sum of observations	
		Hours required to produce complete wilting		Index of toxicity after 5.5 hours		Index of toxicity from sum of four observations		Hours required to produce complete wilting		Index of toxicity after 2.5 hours		Index of toxicity from sum of four observations		Hours required to produce first drooping		Index of toxicity after 24 hours		Index of toxicity from sum of four observations			
		Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B	Strain A	Strain B
Susceptible	Carter's Sunrise	6½	6½	7	7	31	33	6½	6½	7	7	29	25	5½	14	5	2	7	3	67	61
	Sterling Castle	20	20	1	1	14	14	24	24	5	5	22	22	6	0	1	1	3	2	39	38
	Kanora 6	16	16	7	7	24	24	12	14	7	6	25	23	2	5½	8	1	15	3	64	50
	Norton	20	22	2	2	15	13	22	22	6	6	23	23	23	23	1	1	1	1	39	37
	Kanora 11	20	22	2	1	19	15	20	20	2	2	20	19	14	14	1	1	2	2	41	36
	Monumental	8½	8½	7	7	24	26	5½	5½	8	8	26	26	3½	4	7	5	13	8	63	60
Moderately resistant	Ailsa Craig	24	24	1	1	10	10	9	9	6	6	24	23	6	6	1	2	3	4	37	37
	Norduke 3	22	22	1	1	12	16	27	27	2	2	11	15	3½	5½	2	1	5	3	28	34
	Kansas selection 240–1–1	27	20	1	1	8	13	22	22	5	5	20	21	15	20	2	2	3	2	31	36
	Avon	18	20	5	5	23	21	14	14	6	6	26	26	20	20	2	2	2	2	51	49
	Bide's Recruit	20	20	2	2	19	19	15	15	5	5	22	22	27	22	0	1	0	1	41	42
Resistant	Louisiana Pink	29	29	1	1	7	10	14	14	3	3	21	21	----	----	0	0	0	0	28	31
	Kansas selection 220–1–2	24	20	1	1	8	10	24	24	5	5	21	20	31	31	2	1	2	1	31	31
	Kansas selection 205–3	20	20	1	2	14	15	24	24	3	3	21	21	31	31	2	2	3	3	38	39
	Louisiana Red	18	16	2	2	17	19	24	24	1	2	14	15	8¾	5¾	2	3	4	6	35	40
	Kansas selection 205–1–1	20	20	2	2	24	23	10	10	6	6	29	25	24	22	1	2	1	2	54	50
Total		----	----	43	43	269	281	----	----	77	70	354	347	----	----	37	27	64	43	687	671

The results of these two series of experiments show (1) that the excretory products of strain A are somewhat more toxic than those of strain B, and (2) that varieties which show decided resistance or susceptibility to the organism exhibit similar characteristics when subjected to the toxic materials excreted by the pathogene. This is shown much more distinctly in those cases in which wilting did not occur quickly, thus enabling gradations in toxicity to be manifested. In series 2, as later experiments proved, more accurate results would have been obtained had the growth period of the fungus been limited to three weeks or four at the most. When the growth period is extended past three or four weeks, toxic materials, possibly disintegration products, accumulate in such quantities that the plants succumb too quickly for the manifestation of distinct variations in resistance. Moreover, differences in toxicity are much more apparent when actually observed than when indicated by numerical values. (Fig. 1.)

The relation between the response of resistant and susceptible varieties to the toxic materials is even more evident. (Fig. 2.) This is indicated by the results shown in Table 3, in which the index of toxicity of 10 resistant varieties is compared with that of 10 varieties notably susceptible to wilt. Tests duplicating those of series 1 and 2 were repeated with 8 varieties of tomato and similar results were obtained.

TABLE 3.—*Toxicity of the excretory products of strain A of Fusarium lycopersici to 10 resistant and 10 susceptible varieties of tomato* [a]

Resistant varieties	Index of toxicity	Susceptible varieties	Index of toxicity
Marvel	25	Chalk's Early Jewel	38
Marvana	26	Bonny Best	38
Marvelosa	29	Ponderosa	43
Marglobe	37	New Stone	31
Livingston's Beauty	31	Greater Baltimore	46
Norduke 3	28	Carter's Sunrise	67
Louisiana Red	35	Sterling Castle	39
Louisiana Pink	28	Kansas selection 9A	64
Kansas selection 205–3	38	John Baer	45
Kansas selection 220–1–2	31	Monumental	63
Average	31	Average	47

[a] Classification as to susceptibility obtained from reports from field and greenhouse trials.

TOXICITY OF EXCRETORY PRODUCTS TO PLANTS IN BLOSSOMING AND FRUIT-BEARING STAGE

The experiments reported in the preceding pages indicated that the methods first used were not of sufficient delicacy to obtain a fair comparison of the relative toxicity of the two strains or to determine the comparative susceptibility of different varieties to the excretory products of the fungus. To provide a test of greater accuracy, it was thought that modifications might be made—(1) In the plant to be treated, (2) in the material administered, (3) in the technic of introducing the extract into the plant, or (4) in the environment of the plant after treatment.

Rosen (*21*) has shown that the more mature cotton plants do not succumb so quickly to the excretory products of *Fusarium vasinfectum* as do the younger plants. This is in agreement with results obtained

with the tomato when treated with the toxic products of *F. lycopersici*. Hence it was thought that if older plants were used, wilting would not occur so quickly and more accurate comparisons might be made.

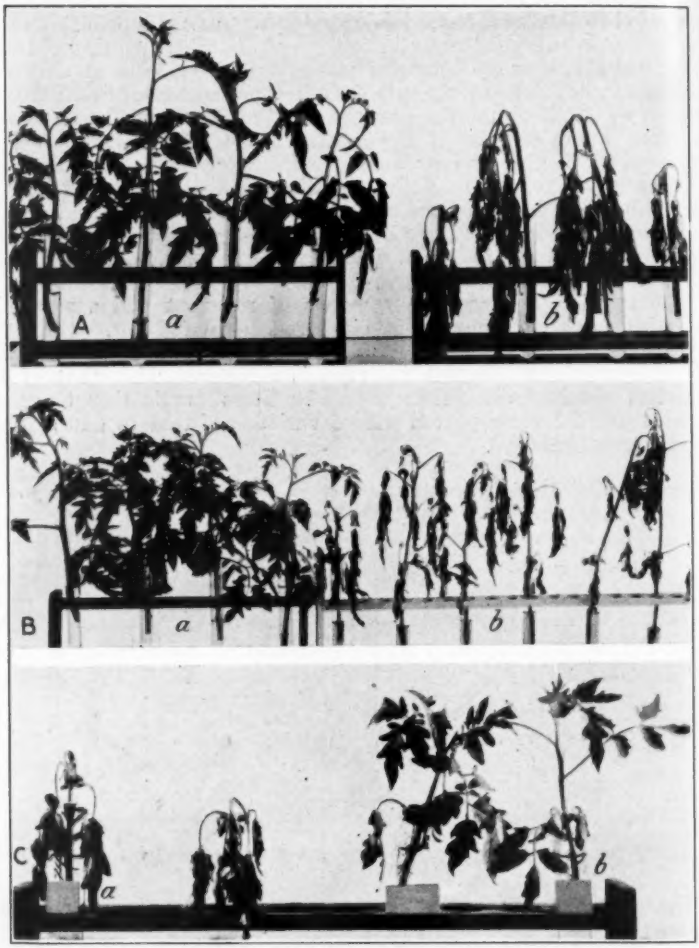

FIG. 1.—A—*a*, Effect of a heavy spore suspension of *Fusarium lycopersici* in water on Marvana seedlings; *b*, effect of the filtrate from a liquid culture of *Penicillium* spp. on Marvana seedlings. B—*a*, Effect of a heavy spore suspension of *F. lycopersici* in Richards' solution on Marvana seedlings; *b*, effect of the filtrate from a liquid culture of *F. lycopersici* on Marvana seedlings. C—Relative toxicity of filtrate from liquid cultures of virulent strain A (*a*) and nearly innocuous strain B (*b*) to Marvana seedlings

Under field conditions the tomato plant is apparently most susceptible to the attack of the wilt fungus after the first blossoms form. In order to simulate natural conditions more closely, a series of 10 experiments was conducted in which plants just beginning to blossom were treated. The means employed in the earlier experi-

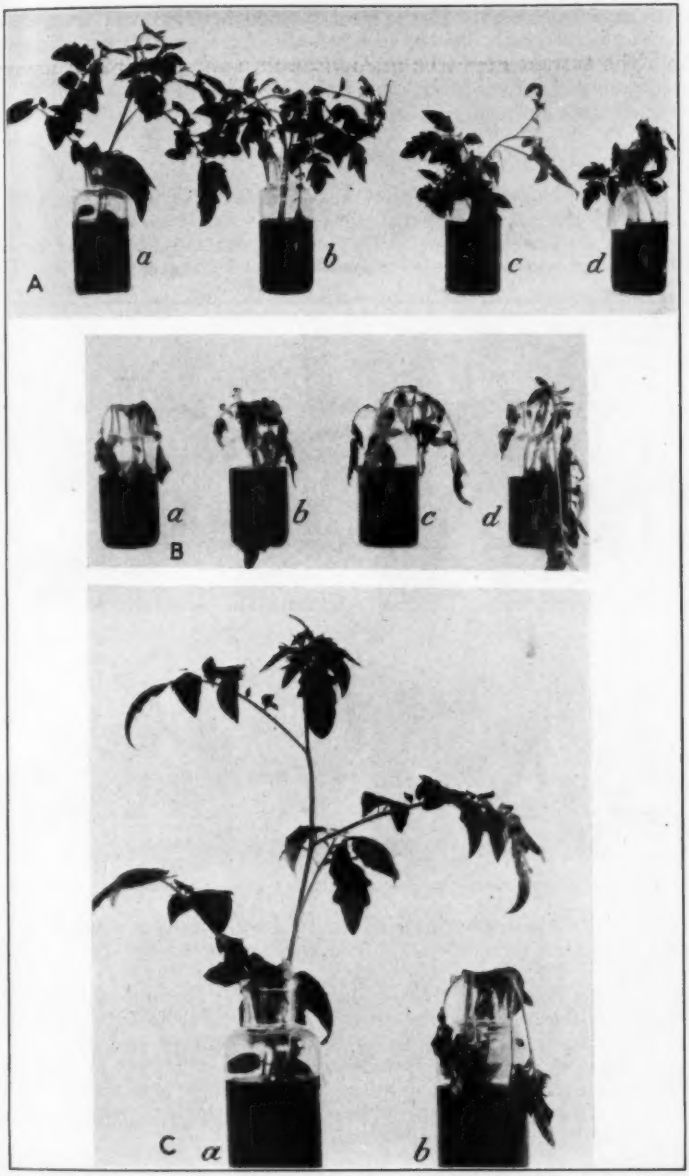

FIG. 2.—Relative susceptibility of seedlings of different varieties to the filtrate from cultures of *Fusarium lycopersici* grown in Richards' solution. A—Reaction of resistant varieties: *a*, Kansas selection 240-1-1; *b*, Kansas selection 220-1-2; *c*, Louisiana Pink; and *d*, Louisiana Red. B—Reaction of susceptible varieties: *a*, Carter's Sunrise; *b*, Monumental; *c*, Kansas selection 9A; and *d*, Avon. C—*a*, Resistant variety Kansas selection 240-1-1 vs.; *b*, susceptible variety Carter's Sunrise

ments in administering the extract seemed far removed from those that would naturally occur, so methods of technic were devised by which the extract might be introduced into the plants in a manner approximating infection by the fungus. The methods used, illustrated diagrammatically in Figure 3, follow.

Experiment 1

The method of stem excision below water was used. As plants had passed the seedling stage, the cut was made at the third node instead of at the soil line. The excised plants were placed in the solutions and were then left in the shade at laboratory temperature.

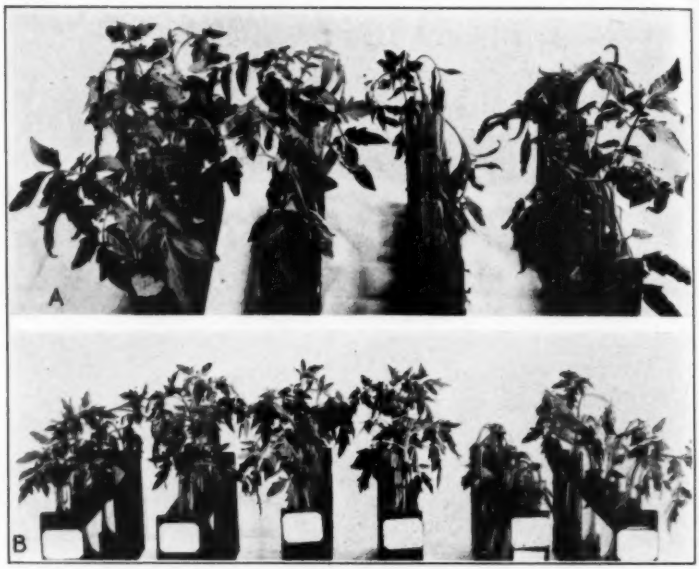

Fig. 3.—Diagrams illustrating methods of introducing toxic materials into tomato plants

That this treatment approached natural conditions somewhat more closely than that formerly employed was evidenced by the fact that the symptoms produced were more nearly like those in the field. Plants of this size when treated do not show drooping of the stem, so characteristic of seedlings treated in a similar fashion, the first indication of a diseased condition being the wilting and collapse of the laminae of the leaflets, followed by the drooping of petioles. While the initial stem drooping of the seedlings is usually closely correlated with the leaf wilting that follows, the latter seems to be much the more significant of the two.

The results of experiment 1 indicate that the products of strain A are more toxic than those of strain B, and that the variety Marvana, which is more resistant to the pathogene than the variety Monumental, is also more resistant to the metabolic products of the causal organism.

Experiment 2

Experiment 2 was a duplicate of experiment 1, except that the filtrate administered was a week older and the plants treated were proportionately more mature than those used in the preceding trial. The results were essentially similar to those previously obtained.

Experiment 3

Plants growing in 7-inch pots were used. The soil was washed away on one side until several of the larger lateral roots were exposed. These were severed below water and the roots to be treated were then introduced into vials containing 35 c. c. of the filtrate. The mouth of the vial was plugged with nonabsorbent cotton to prevent soil from entering. (Note fig. 3, A.)

The wilting which resulted from this treatment resembled that developing in the field. Wilting progressed from the lower to the higher leaves, affecting only those which were supplied by the roots treated. Thus a typical, unilateral wilt, which occurs so commonly under natural conditions, resulted. Individual leaves in some cases, as well as the plant as a whole, exhibited this one-sided wilting.

Experiment 4

The plants were 24 weeks old. They had been grown at a temperature of approximately 19° C. in deep, rich soil in a greenhouse bench. They were especially sturdy, possessing heavy stems and extremely extensive root systems. The treatment was essentially the same as that of the preceding experiment, and the wilting that resulted was comparable to that under field conditions. The abnormal condition was first evidenced by a yellowing, then by wilting and brittling of the lower leaves, followed progressively by the same characteristics in the upper leaves. Following the collapse of leaflets, the petioles began to droop and eventually withered noticeably. Bundle discoloration became so pronounced that it could be detected easily by superficial examination. Repeated attempts to isolate an organism from the blackened bundles were unsuccessful in this and other experiments with larger plants.

The size and vigor of the plants and the comparatively low temperature at which they remained after the treatment resulted in an especially long incubation period, the first reliable indication of the diseased condition not being noticed for 10 days.

Contrary to expectation, it was found to be somewhat difficult to judge the relative toxicity of the products of the two strains, because differences were not so pronounced as often observed when seedlings were treated. A comparison was obtained by noting the number of leaves affected, the extent of their change, the proportion of the plants showing the diseased condition, etc. That this comparison might be as accurate as possible, plants of equal size and vigor were inoculated with the toxic products from each of the two strains. To reduce the possibility of biased judgment on the part of the writer, the readings were taken by disinterested parties, the same criteria for different stages of wilting being used in each case.

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EXPERIMENT 5

The plants were removed from the 6-inch pots in which they had grown. The soil was washed from the lower half of the mass and the roots thus liberated were cut below water and introduced into a vial containing 35 c. c. of the filtrate. The entire mass of upper roots, together with the vial, was replaced in a larger pot partially filled with a sandy soil. Thus the treated plant received the fungal extract through the lower roots at the same time that the uninjured upper roots were functioning normally. (Fig. 3, B.)

EXPERIMENT 6

The plants were so treated that the lower roots and basal portion of the stem could be removed and cut below water without disturbing a mass of upper roots which had developed from subterranean portions of the stem. To obtain this result, the plants were treated as follows: Seedlings in the second-leaf stage, transplanted from flats, were thrust through the openings in 3-inch pots, and the roots and base of the stem were buried in soil in a 4-inch pot. As the plant increased in height, additional soil was placed around the stem in the upper pot in order to induce development of adventitious roots. The plants were kept at a low temperature (18.5° C.) to promote root development at the expense of aerial growth. At the end of 15 weeks the soil was washed from the roots in the lower pot, exposing the extreme base of the stem and the main roots which had developed from the lower portions of the stem. These roots and the base of the stem were then severed under water and placed in vials containing 35 c. c. of the filtrate. The treated plants were placed in the shade at laboratory temperature. (Fig. 3, E, F.)

EXPERIMENT 7

Experiment 7 duplicated 6, except that but one variety was tested and the plants and cultures from which the filtrate was obtained were 1 week older.

EXPERIMENT 8

When tomato seedlings in the fourth-leaf stage were transplanted to 7-inch pots they were so placed that the stems would angle off to one side to facilitate the washing out of dirt from the pot. At the time of treatment the soil was washed from one side of the mass of roots through the opening in the base of the pot. The stem, thus exposed, was cut below water and introduced into a vial containing 35 c. c. of filtrate. The vial was closed with a cork and the soil was replaced in the pot. (Fig. 3, C.) Wilting resulted in a relatively short time, as the plants were left in a high-temperature greenhouse, fully exposed to the sun.

EXPERIMENTS 9 AND 10

In these two experiments the plants were treated in the same manner. The potted plants were placed in pot saucers filled with sand which was watered daily for a period of five weeks preceding treatment. As a consequence, a large mass of fibrous roots grew

through the opening in the pot into the sand in the saucer. After the sand had been washed off the roots were cut below water and immersed in a wide-mouthed vial containing 35 c. c. of the filtrate. After treatment the plant was watered from above, so that wilting would not be induced by a lack of soil moisture. (Fig. 3, D.)

Wilting developed in a much shorter time in experiment 9 than in experiment 10, due probably to the fact that a more susceptible variety was used; and also to the fact that somewhat larger root systems had developed, thus allowing the absorption of greater quantitites of the filtrate before the vessels in the cut ends of the roots had become clogged.

The results of the 10 experiments are summarized in Table 4.

TABLE 4.—*Toxicity of the filtrate from cultures of strains A and B, grown in Richards' solution, to tomato plants of different varieties in the blossoming stage*

[Index of toxicity bassed on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Experiment No.	Tomato variety	Age of plant	Number of plants used	Method by which extract was introduced into plants	Concentration of Richards' solution	Age of culture	Shortest incubation period		Index of toxicity	
							Strain A	Strain B	Strain A	Strain B
		Weeks			*Per cent*	*Days*	*Hours*	*Hours*		
1	Marvana	11	6	Through stem cut off below water (at third node).	50	14	24	28	66	39
	Monumental	11	6	do	50	14	20	20	73	61
2	Marvana	12	6	Through stem cut off below water (at third node).	50	21	9	10	37	32
	Monumental	12	6	do	50	21	7	9	49	41
3	Gulf State Market	17	6	Through lateral roots cut off below water; potted plants.	100	41	38	44	28	14
4	John Baer		12	Through lateral roots; plants in bench.	100	28	240	288	30	24
5	do	15	24	Through basal roots	50	22	48	48	27	22
	do	15	4	do	50			34	12	17
	do	15	4	do	100	14	38		12	0
6	Louisiana Red	15	4	Through excised stem and roots; upper part of root system intact.	50		39	34	18	15
	do	15	4	do	100		38	34	14	16
7	John Baer	16	26	do	100	22	16	17	54	41
8	Norton	15	12	Through excised stem and roots; upper root system partially intact.	100	24	22	22	22	18
9	John Baer	17	32	Through roots projecting through base of pot.	100	20	36	40	65	41
10	Kanora 11	15	12	do	100	35	120	192	50	38
	Total		164						557	419

The results from this set of experiments correspond closely to those obtained with seedlings subjected to conditions favoring extremely rapid wilting. It was found that for purposes of comparison between strain behavior, or the response of different varieties to the toxic materials, the method employed in experiments 1 and 2 was as satisfactory as the others and involved less labor.

For purposes of strain comparison, the best results were obtained when excised stems of more mature plants of a resistant variety were

placed in the filtrate of 2-weeks-old cultures grown in 50 per cent Richards' solution and left in the shade at a temperature of 22° C.

The introduction of the toxic materials through the roots of plants is a slight departure from the more artificial system employed in practically all previous investigations. There is a marked resemblance between the symptons of seedlings affected with Fusarium wilt and those of young plants which have absorbed toxic materials from fungal cultures. This resemblance is more pronounced when the symptoms of true wilt under field conditions are compared with those developing from the introduction of filtrate into the roots of fairly mature plants. This was shown in an especially marked degree in experiments 4, 9, and 10.

RELATION OF TEMPERATURE TO TOXICITY OF EXCRETORY PRODUCTS OF STRAINS A AND B OF FUSARIUM LYCOPERSICI

Repeated studies have been made wherein the ability of certain fungi to cause disease at different temperatures has been compared with their growth rates at those temperatures on some solid-culture medium. When the temperature which favored disease production corresponded to that at which the fungus made its most rapid radial expansion it was assumed that the development of the disease was primarily due to the fact that conditions were favorable for the growth of the fungus. On the contrary, when the two failed to correspond it was usually assumed that disease development was due to some weakened condition of the host which would promote infection. It seemed to the writer in the case of the wilt diseases, at least, that a temperature which might be ideal for the radial spread of a fungal colony on solid media might not be the one at which the fungus would be most pathogenic. It was thought that it might be of value to study the relation of temperature to the production of wilting by toxic fungal excretions. It seemed possible that a correlation in temperature relationships between the optima for disease development and the production of wilting by the toxic materials might be of greater significance than a correlation between temperatures favoring disease development and the lateral spread of the fungus.

It was suggested by Richards (*20*) that there might be two possible explanations for the maximum development of Rhizoctonia cankers on the potato, pea, and bean at temperatures below those which favored lateral spread of the fungus on solid-culture media—(1) that the pathogene might be inhibited by its own metabolic products at the higher temperatures, as suggested originally by Balls (*2*), or (2) that the fungus might secrete enzymes at the lower temperature which would be influential in causing a diseased condition. Richards presented no experimental evidence to support either suggestion. Aside from these observations, the writer is unaware of any reference in the literature in which it has been suggested that temperatures other than those which are the optimum for lateral mycelial expansion in culture might be the ones best adapted to the secretion of toxic materials directly responsible for the instigation of the disease.

In addition to securing further evidence regarding the relation of temperature to the production of the disease, it was thought that some temperature combination might be found that would provide a

favorable test for (1) the comparative toxicity of the excretory products of strains A and B, and (2) the comparative reaction of resistant and susceptible varieties of tomato to the wilt-producing factor. The following possibilities were considered: (1) That the temperature at which the plant was exposed when subjected to the toxic materials might be the one of greatest importance; (2) that the temperature at which the fungus was grown and at which the toxic materials were produced might be the critical one; (3) that a study in which the two factors mentioned were combined might provide evidence of the greatest value.

RELATION OF TEMPERATURE AT WHICH PLANTS WERE TREATED TO TOXICITY OF PRODUCTS OF STRAINS A AND B

The filtrate of 21-day-old cultures of strains A and B grown in Richards' solution at laboratory temperature (21°–22° C.) was obtained by the usual method, and 50 c. c. were placed in each of 48 flasks. The different portions of the filtrate were then placed in constant temperature chambers in which the temperatures ranged from 12° to 32° C. Two hours later seedlings of the varieties Stone and John Baer were cut under water and the stems inserted in the extract in the flasks. The results are shown in Table 5. Similar results were obtained when the experiment was repeated.

TABLE 5.—*Production of wilt at different temperatures by the filtrate of 3-weeks-old cultures of strains A and B of Fusarium lycopersici grown at 22° C.*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Temperture at which toxicity of filtrate was tested (° C.)	Relative humidity in the different chambers	Hours required to produce complete leaf brittling (John Baer variety)		Index of toxicity after 24 hours; sum of observations (John Baer and Stone varieties)	
		Strain A	Strain B	Strain A	Strain B
12	69	----------	----------	0	1
16	61	----------	----------	20	18
20	60	30	----------	43	23
24	74	48	48	37	25
28	60	24	48	54	40
32	85	24	30	55	48

The results of this experiment indicate that both temperature and humidity play important rôles in the production of wilt by the toxic materials formed by the growth of the fungus in liquid culture. The conditions favoring rapid transpiration, viz, high temperature and low relative humidity, apparently cause a rapid absorption of the toxic materials and are thus best adapted to the production of wilt in the plants. Additional experiments demonstrated that humidity, as well as temperature, is an important factor in the development of wilting. Plants in the 12° chamber, in which the relative humidity was 60, wilted more quickly than those exposed to a temperature of 16° C. and a relative humidity somewhat higher. Similarly, plants held in a greenhouse at an average temperature of 26° and a relative humidity of 42 wilted in practically the same time as plants subjected

to a temperature of 32° and relative humidity of 80 and much more quickly than those kept at a temperature of 28° and a relative humidity of 88.

Differences in toxicity between strains A and B were more pronounced at the higher temperatures favoring wilt production than at the lower temperatures.

RELATION OF TEMPERATURE TO PRODUCTION OF TOXIC MATERIALS BY STRAINS A AND B

To determine the relation of temperature to the production of toxic materials, six experiments were conducted in which different varieties of plants and the filtrates from 2, 3, and 4 weeks old cultures were employed. The cultures were grown at 12°, 16°, 20°, 24°, 28°,

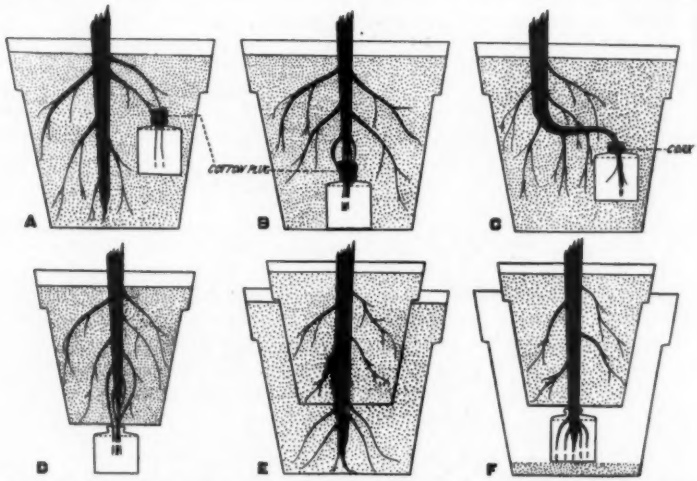

FIG. 4.—Relation of temperature to the production of toxic materials by *Fusarium lycopersici* grown in Richards' solution. A.—Seedlings of Kansas selection 9A in filtrate of liquid cultures grown at the following temperatures (left to right): 20°, 24°, 28°, 30° C. B.—Seedlings of Marvana in filtrate of cultures grown at the following temperatures (left to right): 12°, 16°, 20°, 24°, 28°, 31° C.

and 31° C.; the mycelial mat was removed by filtration in the usual manner and the stems of potted seedlings, cut under water, were inserted in the filtrate. In the first experiment in which 7-weeks-old Marvana and Monumental seedlings were treated, the plants, after insertion in the solutions, were placed in a high-temperature greenhouse at midday fully exposed to the sun. After a period of 60 minutes all of the plants of the variety Monumental showed a definite stem drooping and initial leaf flagging. This was especially marked in those plants in the filtrate of both strains that were grown at temperatures of 20° and above. At the same time all of the plants of the variety Marvana were erect and turgid, with the exception of those in the filtrate of cultures grown at 28°. In the solution of both strains obtained from cultures grown at 28° stems of the plants had drooped completely, so that they resembled those of the susceptible variety Monumental. (Fig. 4, B.) Collapse of the leaflets, which is probably more indicative of true wilt then stem drooping, also

occurred more quickly in plants subjected to filtrate produced at 28° than in those inserted in filtrates produced at other temperatures. This was shown more conclusively in additional experiments in which the plants, after having been cut below water, were placed in the filtrate from younger cultures and were then kept in the shade. (Fig. 4, A.) In a later experiment duplicating this one set of plants was placed in the filtrate of a 28° culture, previously cooled to 16°, and left at that temperature. Meanwhile the plants in the extracts produced at the various temperatures were exposed to an atmospheric temperature of 26° and a low relative humdity. The first plants to droop and wilt were those in the filtrate produced at 28° and held at the higher temperature. The plants next wilting were those in the 28° filtrate in the 16° chamber. Wilting in the plants exposed to 26° in the extracts produced at the other temperatures followed. The results of this experiment are summarized in Table 6.

TABLE 6.—*Production of wilting in tomato seedlings at 22° C. by the filtrate of cultures of strains A and B of Fusarium lycopersici grown in Richards' solution at different temperatures for 28 days*

[Index of toxicity based on the following values: Plant turgid=0; stem drooping=2; leaf collapse=6; leaves water-soaked=8; leaves dry and brittle=10]

Temperature at which fungus was grown (°C.)	Index of toxicity				
	By Marvana and Marvel (varieties resistant to the pathogene)		By Monumental, Stone, and Kansas selection 9A (varieties susceptible to the pathogene)		Total
	Strain A	Strain B	Strain A	Strain B	
12	10	12	19	19	60
16	12	12	58	42	124
20	39	27	77	67	210
24	53	29	84	68	234
28	86	85	123	113	407
31	35	39	63	64	201
Total	235	204	424	373	1,236

These results indicate a definite correlation between temperatures favoring the inception of the disease and the production of materials toxic to plants. There is also a positive correlation between the lateral spread of the fungous colony on solid-culture media and the production of toxic materials. In this connection interesting observations were made as to the condition of the filtrates when grown at different temperatures. The color and density of the liquid were directly comparable to the effects of the different extracts. The filtrate from the 28° C. culture was far darker and more dense than that produced at the other temperatures. The extract obtained from the 24° culture was noticeably darker than those from the 20° and 31° cultures, which were of practically equal density. The filtrates from the cultures growing at 12° and 16° were nearly as clear as the original Richards' solution, which was used as a control. The thickness of the mycelial mat was not so noticeable in the 28° culture as in that grown at 24°, due to the fact that autolysis in the former had pro-

gressed to a more advanced stage. The greater density and deeper color of the filtrates produced at the temperatures best adapted to wilting were due apparently to the accumulation of larger amounts of colloidal material. This conformed with the idea that the metabolic material of highest toxicity is colloidal in nature.

In this series of experiments comparative measurements indicating relative toxicity were made with greater accuracy because the use of younger cultures and the treatment of the plants after the extract was administered provided tests of greater delicacy than those previously employed. The data in Table 6 indicate that the plants of varieties susceptible to the pathogene wilted much more quickly than resistant varieties. In addition it is evident that the metabolic products of the virulent strain A are more toxic than those of the nearly innocuous strain B to susceptible and resistant varieties alike. This was not so marked when plants were treated with material produced at the extreme temperatures, 12° and 31° C. In considering the equal toxicity of the two strains at 31° it may be remembered that strain B made a more rapid radial growth on potato agar than strain A at temperatures above 30°.

RELATION OF TEMPERATURE AT WHICH PLANTS WERE TREATED TO TOXICITY OF PRODUCTS OF STRAINS A AND B FORMED AT DIFFERENT TEMPERATURES

As in the previous experiment, the toxic materials were produced in cultures kept at different temperatures for 35 days. Two hours before the tomato seedlings were treated filtrates produced at the various temperatures were placed in the chambers in which the cultures had grown. Two-months-old Marvana and Monumental seedlings were used for the test.

TABLE 7.—*Production of wilting in tomato seedlings by the filtrate of 5-weeks-old liquid cultures of strains A and B of Fusarium lycopersici at the same temperatures at which the fungus was grown*

[Index of toxicity based on the following scale of values: Plant turgid=0; stem drooping=2; leaf collapse=6; water-soaked leaves=8; leaves dry and brittle=10]

Temperature at which fungus was grown and at which filtrate was held while tested for toxicity (°C.)	Relative humidity of the chambers	Index of toxicity after 24 hours [a]		
		Strain A	Strain B	Total
12	77	8	8	16
16	75	7	7	14
20	44	28	26	54
24	85	27	24	51
28	85	33	32	65
31	85	32	32	64

[a] Results of four trials.

The two previous experiments had shown (1) that if the tomato seedlings were treated with toxic materials produced at the same temperature wilting would be accelerated by a rise in temperature, and (2) that a greater quantity of toxic products, or materials of a higher degree of toxicity, were produced at 28° C. than at the other

temperatures. By producing wilt at the same temperature at which the toxic materials had been formed it was hoped that some information might be obtained as to which temperature factor was the more important—the one influencing the formation of deleterious metabolic products of the fungus or the one that would stimulate wilting after the toxic materials had been administered. The results of the first experiment are shown in Table 7. Experiments duplicating the one recorded produced results essentially similar to those indicated.

From these data it would seem that final wilting is influenced (1) by the temperature which is best adapted to the pathogene and (2) by those temperatures and atmospheric conditions which apparently cause some lack of physiological balance in the treated plant. Practically the same index of toxicity was recorded for the 28°–28° C. and 31°–31° combinations in all of the experiments conducted. The influence of humidity is evidenced again in the apparently greater toxicity of the 20°–20° combination as compared with the 24°–24° combination.

DISCUSSION

If wilting in nature is caused by fungal excretory products it would seem from the results of these three experiments that optimal conditions for the development of the disease would result from that combination of environmental factors best promoting the production of toxic materials by the fungus and at the same time predisposing the plant to natural wilting.

Theoretically, an ideal combination would be a temperature of 28° C. for the greater part of the day, during which time toxic materials would be produced in greatest abundance, followed by periods of higher temperature in which the plant would be more inclined to wilt from the increased rate of transpiration. This would agree with the findings of Clayton (7), who has presented evidence to prove that ideal conditions for the development of wilt are those that combine a temperature of 28° during the greater part of the day with intermittent periods of strong sunlight and higher temperatures. It would also agree with the results of the temperature tests conducted by Clayton as well as the writer, in which it was found that temperatures in the neighborhood of 31°–32° were more favorable for wilt production than a temperature of 24°, in spite of the fact that the latter is more conducive both to the radial spread of the fungus on solid media and to the production of toxic materials.

The most significant fact obtained from the three experiments was that the temperature which is ideal for the development of the disease was the most favorable for the production of wilting. Whether the greater toxicity of the filtrate produced at this temperature is due to a quantitative or a qualitative relationship is undetermined. The greater accumulation of materials, apparently colloidal in nature, suggests that the quantitative factor is of great importance.

STATISTICAL RECORD INDICATING RELATIVE TOXICITY OF EXCRETORY PRODUCTS OF STRAINS A AND B

In most of the experiments dealing with the relation of toxic fungal excretions to wilt the products of both strains were used in order to obtain a comparison of their ability to cause wilting. In the various

experiments conducted a total of 3,316 observations were recorded involving 1,658 comparisons of the relative toxicity of the products of the two strains. The results of these observations may be noted by the statistical record in Table 8.

TABLE 8.—*Statistical record indicating roughly the relative toxicity of the excretory products of strains A and B of Fusarium lycopersici*

Experiment No.	Number of comparisons	Strain A more toxic	Strains A and B of approximately equal toxicity	Strain B more toxic
1 (first varietal test)	192	55	129	8
2 (second varietal test)	425	68	325	32
3 (third varietal test)	163	37	83	43
4 (10 experiments with plants in the blossoming stage)	206	110	68	28
5 (temperature experiments)	672	192	380	100
Total	1,658	462	985	211

The data in Table 8 show that in the majority of cases excretory products from virulent strains are apparently no more toxic to plants than those from practically innocuous strains. When there is an apparent difference, however, the products from the more virulent strain are usually the more toxic. This is indicated more strikingly in experiments in which more delicate tests were made. Thus in experiments 2, 3, and 5, listed in Table 8, in which 5 and 6 weeks old cultures were used, the results do not indicate such distinct variations in toxicity as do those of experiments 1 and 4, in which the methods were improved by the use of younger cultures and older plants.

In later experiments, in which the cultures were grown for two or three weeks in 50 per cent Richards' solution, the products of strain A were practically always more toxic than those of strain B.

SUMMARY AND CONCLUSIONS

A definite correlation was found to exist between the pathogenicity of *Fusarium lycopersici* and the toxicity of its metabolic products. This correlation was expressed in (1) similarity of symptoms (fig. 5), (2) the relation of different strains of the pathogene to wilting, (3) the similar reaction of susceptible and resistant varieties to the pathogene and to its toxic products, and (4) the relation of temperature to wilting. These results provide additional evidence to show that final wilting is caused, at least in part, by toxic materials liberated by the fungus. They also suggest that resistance is influenced to some degree by physiological factors.

When tomato plants in the blossoming stage were treated with the filtrate from liquid cultures of strains A and B of *Fusarium lycopersici* symptoms similar to those occurring in older plants in the field resulted.

Varieties that were resistant to the fungus itself were likewise more resistant to the excretory products of the fungus grown in culture than were varieties strongly susceptible to the fungus. Similarly, the metabolic products of the more pathogenic strain A proved to be more

toxic on the whole than those liberated by the comparatively innocuous strain B.

Temperature and relative humidity were found to be important factors in causing wilting in plants inserted in fungal extracts. Plants subjected to toxic materials produced under similar conditions wilted more quickly the higher the temperature and the lower the atmospheric humidity.

The excretory products from cultures grown at 28° C. proved to be more toxic than those produced at other temperatures. Plants placed in the toxic materials produced at 28° and exposed to a temperature of 28° wilted in practically the same time as those with stems submerged in filtrate produced at 31° and subjected to a temperature of 31°.

FIG. 5.—Wilting of a tomato plant (a) caused by the insertion of filtrate from a liquid culture of *Fusarium lycopersici into the root system*

The predisposition of the tomato to wilt at higher temperatures is apparently due to a combination of factors: (1) The vigor of the pathogene at temperatures ranging from 24° to 30° C.; (2) the fact (which is probably of greater importance) that at those temperatures the fungus produces excretory materials of greatest toxicity to the host plant; (3) at temperatures exceeding 30° the unbalanced physiological condition of the host plant renders it more subject to the action of the toxic products. This is probably a factor of great importance, since temperatures of 30° to 32° are more conducive to wilt production by the pathogene than those around 24°, in spite of the fact that the fungus grows more luxuriantly and produces materials of greater toxicity at the latter temperature.

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SEX RATIOS IN CUCUMBER FLOWERS AS AFFECTED BY DIFFERENT CONDITIONS OF SOIL AND LIGHT [1]

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INTRODUCTION

Sex ratio in plants has been studied in some detail; sex ratio in flowers of different monoecious plants has received less attention; and no available research on the ratio of flowers of *Cucumis sativus* L. has come to the writer's attention. Because of the economic importance of greenhouse cucumbers, their behavior when grown under varying conditions of environment, as observed in commercial houses, suggested the advisability of determining the cause of some of the peculiar phenomena associated with a particular variety grown during the summer and winter months when light conditions vary widely.

Observations in commercial greenhouses where cucumbers are grown reveal the fact that cucumbers of different varieties vary in their ability to set fruit at various times during the year, some varieties setting much more than others. The cause of extreme prolificacy is undoubtedly associated with poor shape of fruit. Because of the effect which growing conditions have on the shape of fruit and the relation of these conditions to the location of pistillate flowers on the plants, an extensive study was undertaken to determine, if possible, the cause of many of the physiological and morphological phenomena which seem to be more or less closely related.

Because of the variability of genetic selections when grown during days of maximum and minimum sunlight, it was deemed advisable to determine, if possible, the effect that varying any one factor would have on the behavior of the selections, and thus establish the reliability of data collected on these selections at any one time of the year.

Cucumbers are monoecious in flowering habit, and cross-pollination is the rule. This has resulted in varieties becoming extremely heterozygous. When these varieties are selfed many different types of cucumbers may be isolated.

The fact that the Granite State variety produces a heavy "set" of pistillate flowers on the nodes of the main stem while the Belleville variety produces very few, if any, pistillate flowers on the nodes of the main stem suggests that this character at least is controlled to a certain extent by one or more genetic factors. Accepting this

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as a hypothesis, a genetic analysis of these varieties was begun, but as yet sufficient data have not been collected to show satisfactorily the relation of chromosomes to the location of pistillate flowers.

SELF-POLLINATION STUDIES AND OBSERVATIONS

The first attempt to solve some of the difficulties of variable "set" was made by self-pollinating flowers on plants showing variations within the varieties. In the second generation, various types of both fruit and foliage characters were isolated, some of which were homozygous for certain characters, as was proved by later tests, while others again broke up in the third generation. The location of pistillate flowers was studied in detail.

A number of pure-line selections were isolated and given numbers. Among these, the selections listed below were especially interesting. These were grown during the days of rather short daylight and blossomed from January 1 to May 30, 1925. Observations on commercial varieties grown during this period show a heavy production of pistillate flowers.

21–3.—A strain bearing 2 pistillate flowers to 1 staminate flower. A very heavy set of fruit on the nodes of the main stem was followed by a large number of nubbins. Although this was not a homozygous pistillate type, it was considered a female type in later experiments. Comparatively few staminate flowers were produced.

80–4.—A normal behavior strain bearing flowers in the ratio of 1 pistillate to 6 staminate; a few pistillate flowers on the main stem.

28–4–1.—A strain bearing 1 pistillate flower to 9.5 staminate flowers. This type was referred to as a male strain. All pistillate flowers were borne on the laterals. Staminate flowers were abundant on the main stem.

C29–12.—A strain producing all pistillate flowers, many of them grown together and fasciated. Some of the nodes had 14 pistillate flowers in a cluster.

C29–10.—A normal flowering type but having a bushy habit of growth with many heavy-stemmed more or less stiff branches growing erect.

70–6.—A dwarf or short-stem strain producing only pistillate flowers, the fruit being white and short. Very few, if any, lateral branches were produced.

72–1.—A strain similar to 70–6 except that the fruit was green.

The isolation of these types grown under similar conditions indicated hereditary factors controlling flower formation and location. During the early part of the life cycle of the plants the character for flower formation seemed very flexible, while later in the life cycle when the number of staminate flowers decreased the plants became quite fixed for the pistillate condition. This was generally the case with those strains in which a heavy production of pistillate flowers occurred on the nodes of the main stem. Strains which produced only pistillate flowers late in the life cycle under maximum light conditions, under minimum light conditions tended to become stunted, and numerous fasciated pistillate flowers subtended the lateral branches and main stem.

A decided difference in vigor was noted between various selections. Most of the selections were made in second-generation plants from self-pollinated seed and were found to be homozygeous for characters under observation. In height some selections were uniformly 75 cm. as compared to adjacent selections which were uniformly 2½ meters. Among the heterozygous types the plants ranged from 75 to 200 cm. in height. These show the normal type of growth. The dwarf types had short internodes with many lateral branches. The bushy types had many erect laterals and grew to a height of 1 meter, whereas a

vigorous normal type grew to a height of 3 meters. This difference in height was due to a difference in length of internode as the number of nodes was comparable for the various selections. These selections were made under comparable conditions of environment. How the selections would react to varying conditions of light has not been studied in all its aspects, but a few suggestions will be made later. Some of the selections did not noticeably decrease in vigor up to the sixth generation of selfing.

REVIEW OF LITERATURE

The review of literature in other publications is so complete that only those papers bearing on the monoecious type in plants and those relating to pertinent physiological problems have been considered in the preparation of this article.

There are many references to the general problem of sex expression in plants, and it was deemed unwise to try to harmonize the conclusions, other than those having some application to the monoecious type, with those presented later in this paper.

There is a group of investigators of sex inheritance who recognize only genetics as the determining mechanism, another group who claim that environment is the basis of sex determination, and a third, who take an intermediate position and consider the problem from several points of view.

Among those investigators who attribute the expression of sex in plants to physiological relations, which are subject to change by variations in environment, may be mentioned Burbidge (*10*),[3] Camus (*11*), Castle (*12*), Clute (*13*), Correns (*14*), Davey and Gibson (*16*), Fujii (*18*), Gardner (*19*), Georgeson (*20*), Guinier (*21*), Irmscher (*23*), Meehan (*28, 29*), Nagai (*31*), Riede (*32, 33*), Schaffner (*34, 35, 36, 37, 38*), Stout (*40*), and Yampolsky (*41, 42*).

Among those who would associate chromosomes with sex expression many be mentioned Allen (*1, 2*), Blackburn (*4, 5*), Cummings and Jenkins (*15*), and Janchen (*24*).

Blaringhem (*6, 7, 8*), Emerson (*17*), and Sharp (*39*) show that sex is fundamentally hereditary in nature but concede that environment has its effect in the expression of the sexual state.

Some workers base their conclusions on general observations rather than on controlled experiments, so that many of those in the first group may be classified differently on further investigation. In the first group are a few who emphatically deny the possibility that heredity plays any part in the determination or expression of sex in plants. Because of the large number of species on which observations have been made and from which conclusions have been drawn without first determining whether sex characters could be established as homozygous or heterozygous states, it seems reasonable to suppose that contrary observations might result.

If sex is determined by allelomorphic genes, as has been suggested by Emerson (*17*), it seems especially important that the genetic constitution of material used for environmental studies be definitely established. Thus, if the factors are in a homozygous condition they would respond less readily to changes in environment than if they were in a heterozygous or weakened condition, and would be more or

[3] Reference is made by number (italic) to "Literature cited," p. 744.

less in harmony with Janchen's (*24*) suggestions that weaknesses do exist in certain individuals.

EFFECT OF LIGHT ON FLOWER PRODUCTION

Because of the importance of the location of pistillate flowers in greenhouse cucumbers and its relation to growing greenhouse cucumbers under minimum light conditions, a study of the relation of environment was made.

Selection C29–12, a homozygous pistillate type which produced only pistillate flowers under unfavorable light conditions and a high proportion of pistillate flowers under optimum conditions, was used in studying the effect of light and nutrients on set of fruit. C29–12 is a selection of the Granite State variety. Though a selfed strain, it had sufficient vigor to be used for this work. Space did not permit the use of more than two selections in the first experiment, namely, C29–12 and 28–4–1, and these were employed in later studies.

EXPERIMENT 1

METHOD[4]

The seed was sown in flats in good garden soil on March 1, and when the first true leaf appeared the plants were transferred to 10-quart wooden tubs. Forty tubs were included in each of three series. Twenty of the tubs were filled with a fertile garden soil. After the plants were well started, light applications of nitrate of soda in solution were given every two weeks to keep them growing vigorously. The second lot of 20 tubs of each series was planted in a soil consisting of one-half sand and one-half garden soil by volume. No nitrogen was added to these 20 plants in each series. Series 1 received regular sunlight; series 2 received regular sunlight and also electric light from 2 a. m. to sunrise. Four ordinary 200-watt Mazda lamps were used. Series 3 was shaded from the direct sunlight by a light brown wrapping paper supported by cheese cloth. The plants were grown on strings attached to wires 6 feet above the tubs. Laterals were pinched back to three nodes. Daily records of the flowers were kept, and every pistillate flower was pollinated from another plant within the series. The plants began to flower April 26 and the experiment was discontinued June 21. Pistillate flowers which opened were counted separately from those which were formed but dried up before they opened.

RESULTS[5]

In Table 1 the summarized data show an increase of staminate flowers in the electric-light series over those in the control on fertile soil, where the plants grew vigorously to the end of the experiment at a time of the year when practically the maximum amount of sunlight was available. In the shaded series the total number of pistillate flowers per plant was reduced over 40 per cent, while the total number of staminate flowers was increased 7.7 per cent. The wide ratio of 26 staminate flowers to 1 pistillate flower is due pri-

[4] Unless otherwise stated, the method was the same in all of the experiments.

[5] In all figures and graphs the data have been corrected to 12 plants unless otherwise stated, because the average for each plant would not give sufficient data for good graphs.

marily to a decrease in pistillate flowers. In the case of the series receiving additional electric light, the increase in the ratio over the control (17 to 1 as compared to 14 to 1 for the control) is due to an increase in total number of staminate flowers. Among the plants grown in a soil low in nutrients the number of staminate flowers hardly varied in the three series, but the number of pistillate flowers was slightly smaller in the shaded series and in the series receiving electric light than in the control. The total number of both staminate and pistillate flowers was less on plants grown in unfertile soil than on those grown in fertile soil.

TABLE 1.—*Average number of staminate and pistillate flowers produced on cucumber plants not exposed to direct rays of the sun; on plants exposed to normal sunlight and to electric light from 2 a. m. till sunrise; and on plants exposed only to normal sunlight; grown in fertile and unfertile soil from March 8 to June 21*

FERTILE SOIL

Treatment of plants	Average number of flowers per plant		Number of staminate flowers to one pistillate	Average number of dried pistillate flowers per plant	Average number of flowers unopened at end of experiment		Average number of pistillate flowers per plant
	Staminate	Pistillate			Staminate	Pistillate	
Received normal sunlight (control)	174.5	12.3	14.2	54.0	12.4	18.9	85.2
Received normal sunlight and also electric light from 1 a. m. till sunrise	210.5	12.4	17.0	46.3	12.8	32.9	91.6
Shaded; no exposure to direct rays of sun	188.0	7.1	26.5	29.5	35.3	22.5	59.1

UNFERTILE SOIL

Normal sunlight (control)	136.5	9.4	14.5	28.2	2.3	8.9	46.5
Received normal sunlight and also electric light from 2 a. m. till sunrise	136.5	7.8	17.5	25.5	1.8	3.7	37.0
Shaded; no exposure to direct rays of sun	138.5	8.8	15.7	20.9	4.3	2.6	32.3

In Figure 1, C29–12 and 28–4–1, two self-pollinated selections, and C100, a hybrid (Belleville variety), are compared for flowers. These plants were grown side by side in a greenhouse bed from February 1 to April 30. The ratio of 2 staminate flowers to 1 pistillate flower for C29–12 is considerably lower than the ratio secured from the experiment (14.2 staminate : 1 pistillate) during a period of days having more intense sunlight for a longer period each day. Comparing the average number of flowers for each plant, the narrow ratio in Figure 1 (2 staminate : 1 pistillate) is due to a decided decrease in staminate flowers and an increase in pistillate flowers. Thus a difference in amount and intensity of light seems to have a decided effect on the production of flowers. A difference of 30 calendar days has a marked effect on the plants because of the difference in the amount of light which they receive at a time when they are forming flower primordia. In Figure 2 a comparison of the control with the electric-light series is shown graphically for the duration of the experiment. Electric light increased the production of staminate flowers (electric light 17 staminate : 1 pistillate; control 14.2 stami-

nate : 1 pistillate) during the latter part of the life cycle of the plants. The effect during the first part of the cycle was not so noticeable. The difference noted suggested growing plants from the same lot of seed during days of reduced sunlight.

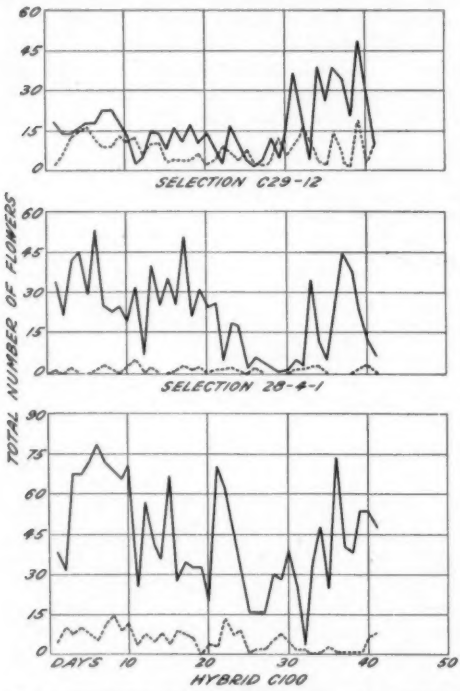

FIG. 1.—Comparison of two selfed selections and a hybrid for staminate and pistillate flowers; pistillate flowers are shown by dotted lines; staminate flowers by solid lines. Data were taken February 1 to March 15

EXPERIMENT 2

METHOD

As in experiment 1, the plants in experiment 2 were grown in three series. 1. Control plants. 2. Plants receiving regular sunlight and electric light. The lights were turned on at 2 a. m. and allowed to remain on until sunrise. On cloudy days they were lit until 6 p. m. The lights were placed 5 feet above the plants, and as the plants grew higher they were gradually raised. The intensity of the light at the top of the plants was 100 foot-candles, which reduced the amount of steam necessary to keep the compartment at 65 F. during the night. No difference in the air temperature was noticeable between this series and the control. 3. Plants placed on a truck were run into a dark chamber at 3 p. m. and taken out at 9 a. m. In each of the three series half of the plants were grown in poor soil, and the other half in very fertile soil.

RESULTS

In Table 2 a summary of the data obtained in experiment 2 is given. The ratios here shown for control plants agree very favorably with the ratio for C29–12 in Figure 1. The total number of staminate flowers for each plant is considerably lower than for the same selection as shown in Table 1, where the data are for plants grown under maximum light conditions. The number of pistillate flowers was very much the same. Thus a reduction in the intensity and duration of the daylight decreased the number of staminate flowers.

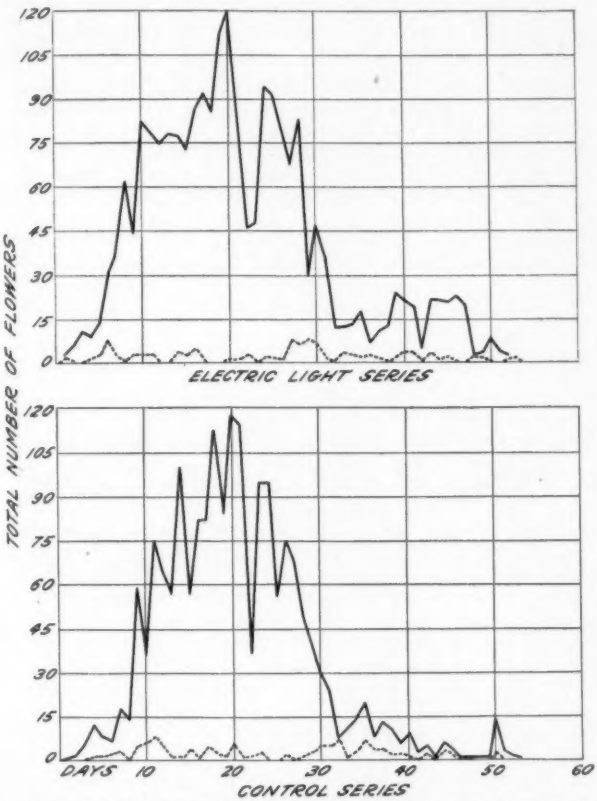

FIG. 2.—Behavior of a strong pistillate flower selection, C29-12, for staminate and pistillate flower production with and without additional electric light, when grown during days of relatively long sunlight. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

TABLE 2.—*Average number of staminate and pistillate flowers produced on selection C29–12 when plants were shaded from 3 p. m. to 9 a. m.; when they received normal sunlight and also electric light from 2 a. m. to sunrise and on cloudy days; and when they received sunlight only; grown in fertile and unfertile soil from December 1 to March 1*

Treatment of plants	Fertile soil			Unfertile soil		
	Average number of flowers per plant			Average number of flowers per plant		
	Staminate	Pistillate	Number of staminate flowers to one pistillate	Staminate	Pistillate	Number of staminate flowers to one pistillate
Sunlight only (control)	16.4	12.9	1.3	14.0	9.3	1.5
Normal sunlight; electric light from 2 a. m. to sunrise and on cloudy days	37.5	13.0	2.9	37.0	10.6	3.5
Shaded from 3 p. m. to 9 a. m.	4.2	3.7	1.1	10.2	4.0	2.6

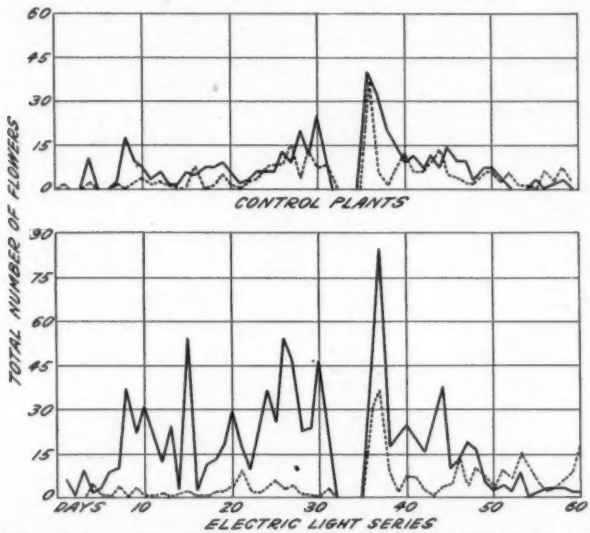

FIG. 3.—Effect of electric light on the production of flowers on plants grown during days of minimum sunlight. The gap after the thirty-second day was caused by naphthalene fumigation which prevented the flowers from opening. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

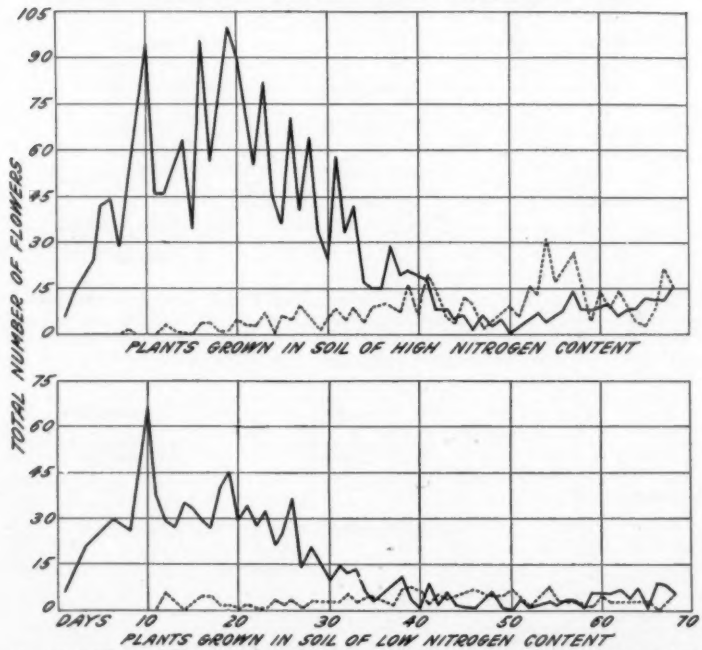

FIG. 4.—Effect on the production of pistillate and staminate flowers of growing plants in soil of high and low nitrogen content during days of relatively long sunlight. Dotted lines show daily production of pistillate flowers; solid lines show daily production of staminate flowers

Comparing the electric-light series with the control series, a material increase is noted in the production of staminate flowers in the electric light series, as is shown graphically in Figure 3. Shading the plants reduced both staminate and pistillate flowers. (Table 2.) The fertility of the soil increased the number of pistillate flowers and slightly reduced the ratio. The greatest difference is shown in the shaded plants, where more staminate flowers were produced.

EFFECT OF FERTILITY OF SOIL ON FLOWER PRODUCTION

In these studies a fertile soil is discussed in terms of nitrogen even though the specific effect of nitrogen in every case is not known.

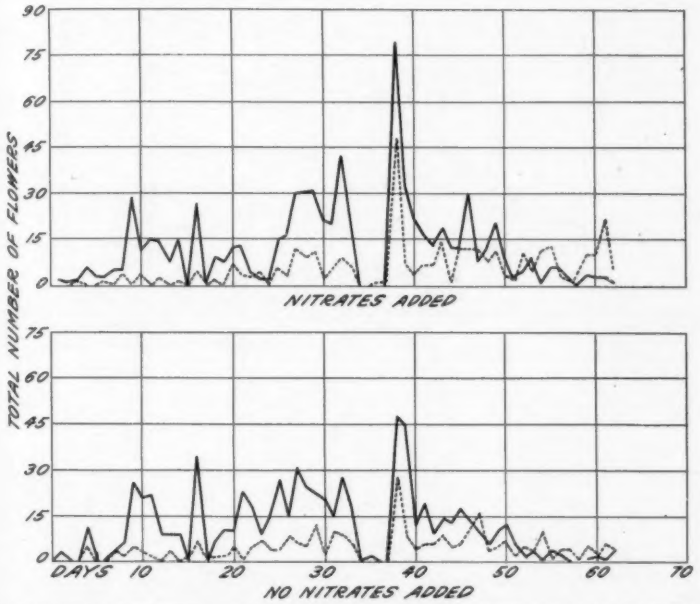

FIG. 5.—Effect on the production of pistillate and staminate flowers of growing plants in soil of high and low nitrogen content during days of minimum sunlight. Dotted lines show daily production of pistillate flowers; solid lines show daily production of staminate flowers

The common observation is that if cucumber plants become yellow and nitrogen is used in one of five different combinations KNO_3, $NaNO_3$, $(NH_4)_2SO_4$, urea, $(NH_4)_3PO_4$, the effect is to cause the plant to become quite green, the degree of color depending on the amount added. Too much at one time causes burning at the tips of the leaves.

In Figures 4 and 5 the effect of growing plants in a soil of high nitrogen content is shown. During the days of maximum sunlight there was a total increase in staminate flowers on the plants grown in high nitrogen soil of 45.5 per cent, and an increase in pistillate flowers of 55.08 per cent. During the days of minimum sunlight there was an increase in staminate flowers of only 3.14 per cent, but in pistillate flowers there was an increase of 20.67 per cent. A com-

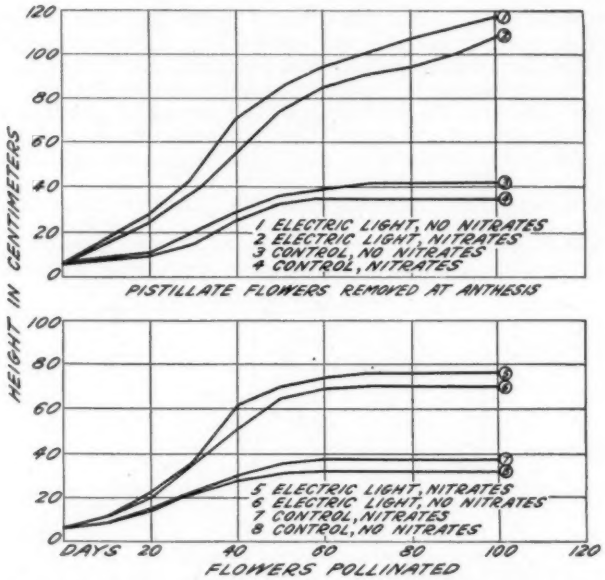

FIG. 6.—Growth rate of plants receiving normal sunlight and electric light or normal sunlight only (control), grown in soil with and without nitrates during days of relatively short sunlight. The upper set of curves present data for plants from which the pistillate flowers were removed at anthesis; the lower set present data for plants on which all pistillate flowers were pollinated

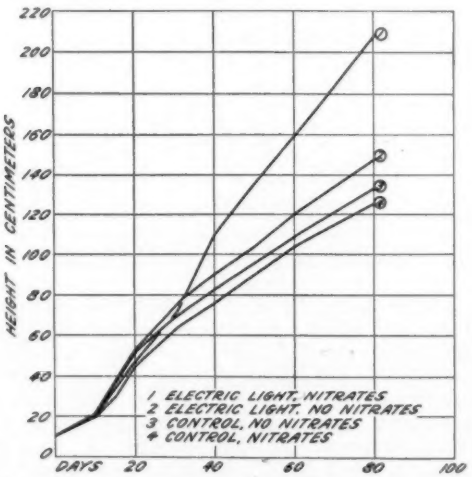

FIG. 7.—Growth rate of plants receiving normal sunlight and electric light or normal sunlight only (control), grown in soil with and without nitrates during days of relatively long sunlight

parison of Figures 4 and 5 indicates that the utilization of nitrogen is dependent on the light increment or sugar content of the plant.

In Figures 6 and 7 growth curves for these plants are shown. Figure 6 shows the effect of light and nitrogen during the days of relatively short sunlight. The wide difference in final height of plants is indicative of the important effect which a small increase in light exerts during the short days. The control plants stopped growing when 32–37 cm. high and produced a cluster of pistillate flowers at the tip with no lengthening of the internodes.

Plants grown under days of relatively long sunlight showed a much more rapid rate of growth, and only where nitrogen was added did

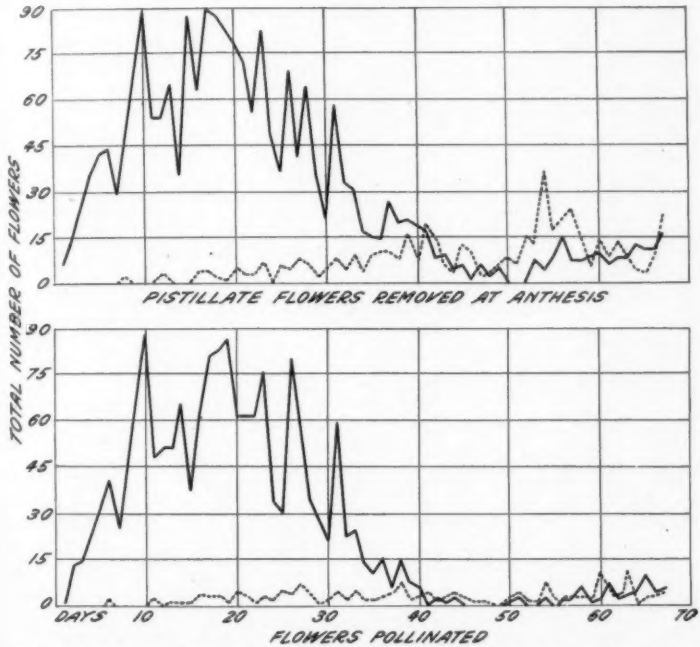

FIG. 8.—Effect of removing all pistillate flowers at anthesis on the later production of pistillate and staminate flowers. These plants were grown during days of relatively long sunlight. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

the additional electric light cause a significant increase in rate of growth. These growth curves are the result of data taken on the main stems of the plants. It is interesting to note that even though the plants grown in soil of high and low nitrogen content differed widely in appearance, the rate of growth of their main stems was similar.

EFFECT OF DEVELOPING FRUITS ON THE RATIO OF STAMINATE TO PISTILLATE FLOWERS

The production of new flowers on plants soon after a mature fruit was picked suggested an experiment in which the pistillate flowers on some plants were removed at anthesis, while other plants were per-

mitted to mature any pistillate flowers which opened. No definite number of fruits was left on the plants because a plant regulates the number that it will carry. There are always more pistillate flowers produced than the plant can successfully mature. If the pistillate flowers are all pollinated and become fertilized, the first ones will start to grow and establish an inhibitory effect on those pollinated later. The extent to which this inhibitory effect is manifested differs in different plants. One plant, for example, may develop only one fruit at a time, while another, very similar in manner of growth, may develop five or six. Flowers that are pollinated simultaneously usually grow equally well.

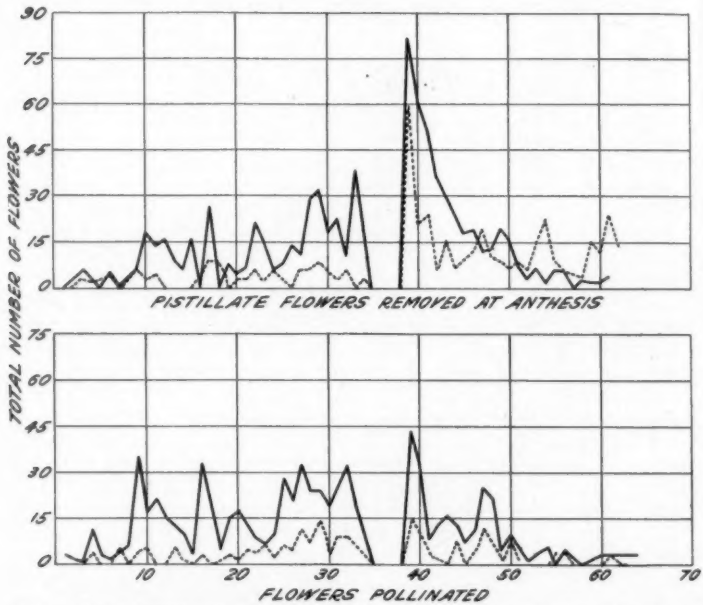

FIG. 9.—Effect of removing all pistillate flowers at anthesis on the later production of pistillate and staminate flowers. These plants were grown during days of minimum sunlight. Pistillate flowers are shown by dotted lines; staminate flowers by solid lines

Pistillate flowers which have been fertilized but inhibited from growing because of one or more developing fruits may remain inactive for as long as 30 days or until the growing fruits have been removed, and may then resume growth and sometimes develop into very well-shaped fruits. Usually, however, early inhibition results in poorly shaped fruit because of the maturity of some of the tissue, which turns yellow soon after the cucumber begins to grow. It is not uncommon to find the growth cycle of a fruit interrupted two or three times before it reaches maturity.

In Table 3 data are given for plants with and without fruit grown from April to June, and in Figure 8 the results are shown graphically for the same plants. The removal of the flowers at anthesis in every case increased the production of pistillate flowers and reduced the ratio of staminate to pistillate flowers at least 40 per cent (control 7.9

staminate : 1 pistillate, pistillate flowers removed 3.9 staminate : 1 pistillate). In Figure 9 the effect of removing pistillate flowers on the subsequent production of flowers is shown for plants grown during days of minimum sunlight. The differences shown are similar to those in Figure 8. The pistillate flowers increased 56 per cent while the staminate flowers increased only 8 per cent. The plants were 100 per cent pistillate at the end of the experiments.

TABLE 3.—*Average number of staminate and pistillate flowers produced on plants of selection C29–12 when fruits were allowed to remain on vines and when they were removed; plants grown in fertile and unfertile soil from April to June*

Treatment of plants	Fertile soil			Unfertile soil		
	Average number of staminate flowers	Average number of pistillate flowers	Number of staminate flowers to one pistillate	Average number of staminate flowers	Average number of pistillate flowers	Number of staminate flowers to one pistillate
Shaded:						
Fruit left on [a]	113.0	8.3	13.6	96	5.5	17.4
Fruit removed	160.5	18.0	8.9	103	8.9	11.6
Sunlight and electric light:						
Fruit left on	79.8	7.8	10.2	80	6.5	12.3
Fruit removed	94.0	25.3	3.7	69.4	11.6	6
Normal sunlight (control):						
Fruit left on	99.1	10.8	9.2	68.7	5.3	13
Fruit removed	107.0	25.8	4.1	72.8	14.0	5.2

[a] All pistillate flowers were pollinated on plants carrying fruit.

In Figure 8 the cyclic production of flowers is shown. The general tendency of the plant under optimum light conditions is to produce a large number of staminate flowers early in the flowering cycle, and as the pistillate flowers are fertilized and the fruit begins to grow, the production of staminate flowers practically ceases. As the fruits are removed when mature (not ripe), flowering begins again, but few staminate flowers are produced, with the result that the later pistillate flowers are improperly fertilized and the fruit does not develop well.

Before this fact was known, considerable difficulty was experienced in securing hand-pollinated or controlled fertilized fruit for genetic studies. During days of long sunlight, unless controlled pollinations could be made on the first pistillate flowers, the practice was to remove all pollinated fruit before making any controlled hand pollinations. During days of short sunlight the removal of all fertilized fruits on such selections as C29–12 resulted in the production of practically all pistillate flowers on the new growth, so that self-pollinations were impossible. These plants must be started in a rather unfertile soil and the first pistillate flowers utilized in order to get self-fertilized seed. The use of electric lights will facilitate matters somewhat because more staminate flowers will be produced.

EFFECT OF ENVIRONMENT ON THE APPEARANCE OF THE PLANTS

When a cucumber plant is grown in a pot under favorable light conditions it soon uses its available nitrogen and begins to turn yellow. The leaves are small and an abundance of very small staminate flowers make their appearance at the nodes of the main

stem, and if any laterals have been formed they will have clusters of staminate buds which may or may not open. The laterals remain short, even though four or five nodes have been formed. The internodes are very small in diameter and quite yellow, and the clusters of numerous staminate buds are conspicuous because of their very short pedicels. The plant produces only sufficient sustaining tissue to support the flower buds. Whether these buds will open depends on the amount of nitrogen available to the plant. In the last stages of nitrogen starvation the nodes at the tip of the main stem remain active while the leaves on the lower nodes turn a greenish yellow and finally become quite white, after which they soon become desiccated. Finally the plant reaches the condition shown by the two in Figure 10, A, *a* and *b*. An occasional pistillate flower may be produced at the tip. When the plant can not get its nitrogen from the soil it seems to draw on any surplus in the larger leaves and to transport it to the growing tip, where pistillate flowers may be formed even though the new leaves are very small.

If some form of soluble nitrogen such as urea is added to the soil, a noticeable change in the plants is visible in 24 hours, and in 12 days this results in a condition such as that shown by the two plants in Figure 10, A, *c* and *d*. The older leaves do not become green and resume growth, but gradually turn hard and stiff, while the new leaves assume the appearance of those of a plant receiving sufficient nutrients for luxuriant growth. The new stem becomes large and rugged in appearance while the old stem remains small and yellow. Even though the stem at the base remains small no ill effects from lack of water in the new growth are shown. Figure 10, A, *d*, shows a cucumber developing normally from a flower which has been pollinated previous to the application of urea.

Under reduced light conditions the effect of nitrogen is considerably different on plants producing abundant pistillate flowers on the main stem. In place of the continuation of normal growth, no appreciable extension of the main stem results, but pistillate flowers are thrown out from all nodes, even where dried leaves have been removed. In Figure 10, B, sections of the main stem from a green and a white-fruited plant of the heavy main stem "set" type are shown. The leaves are small, rather long, with very short petioles, which gives them a dwarfed appearance. Under optimum light conditions the fruits on the plant shown in Figure 10, B, *b*, would have prevented the further development of pistillate flowers. Under reduced light conditions the presence of developing fruits has little effect, and large numbers of pistillate flowers are formed even though they seem to be sterile. The tip of the stem becomes a mass of pistillate flower buds and the new internodes become shortened. Theoretically, it would seem as though all fruit tissue and no stem tissue would be the final result. Even though the differentiation of parts take place, development of those parts is handicapped by a lack of some-

EXPLANATORY LEGEND FOR FIG. 10

A.—*a* and *b*, plants which show nitrogen starvation; *c* and *d*, plants which show the response to nitrogen 12 days after the application of urea.

B.—Sections of the main stems of, *a*, a white and, *b*, a green-fruited cucumber plant, showing the effect of reduced light on the growth of leaves and increase in pistillate flower production.

C.—Parthenocarpic fruits developed under reduced light on a pistillate selection which will not develop parthenocarpic fruits under optimum light conditions. The small fruit shows partially developed ovules. Sometimes the seed coat is formed as in a fertilized fruit, but it is always empty.

D.—Fruits similar to those shown in C; *a*, seed cavities.

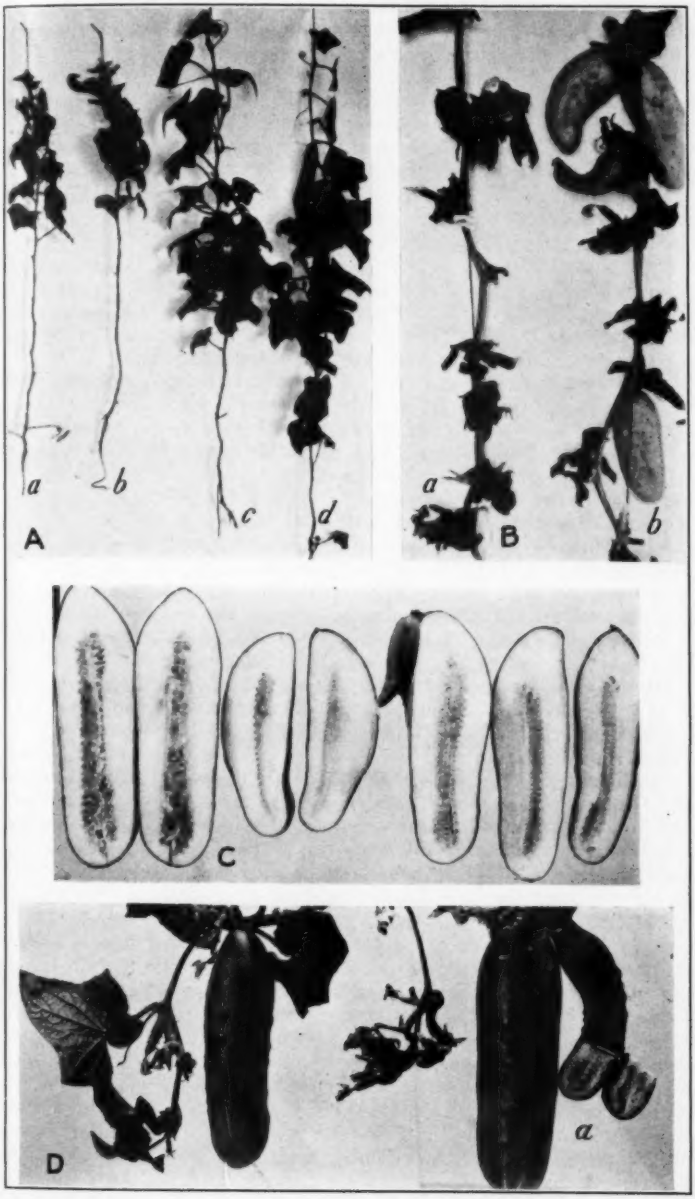

FIG. 10.—*Cucumis sativus*
(For explanatory legend see p. 734)

thing, undoubtedly carbohydrates, for either cell-wall formation or elaboration of proteins. Under these conditions, of which the plants in Figure 10, B, are the result, a false parthenocarpic condition is brought about. Some seeds developed in the three large fruits as a result of an earlier light effect, but the smaller fruits were not pollinated. When the pistillate flowers are pollinated, fertilization does not seem to be accomplished even though good viable pollen is used. Apparently insufficient sugars are available to feed the pollen tube in its growth into the stigma. Brink (*9*) has shown that a rather high concentration of sugar is necessary to bring about germination of the pollen grains. It is not uncommon to find well-shaped fruit being produced without fertilization on such vines under unfavorable light conditions, as is shown in Figure 10, C and D. Under high nitrogen and optimum light conditions, plants from selections like C29–12 produce only one pistillate flower at a node, and unless these are pollinated they immediately dry up after anthesis.

In Figure 11, A, some forms of fasciation found on "pistillate" plants under reduced light conditions are shown. All stages from fasciation of the petioles of staminate flowers to so-called double ovaries may be found, though it must be understood that not all pistillate plants grown under reduced light produce fasciation. The short petioles are characteristic.

To say that this fasciated condition is due entirely to light or entirely to nitrogen would seem unreasonable. Under reduced light conditions a given quantity of nitrogen will keep the plant green for a longer period than under optimum light conditions. Even though a certain amount of nitrogen is necessary for the growth of the plant, yet the available sunlight, quantitatively and qualitatively speaking, seems to be the controlling factor in the abnormal prolificacy of some strains of cucumbers and the false parthenocarpic production of fruits.

The presence of a maturing fruit may influence the reaction of the plant to light. As has already been shown, the removal of the pistillate flowers at anthesis results in the subsequent production of a greater number of pistillate flowers. In Figure 11, B, is shown a plant from selection 90–1–3 which had four pistillate flowers pollinated on the same day and all four of the resultant fruits were permitted to ripen. The plant produced few laterals and the main stem had grown 230 cm. when the picture was taken. A plant (fig. 11, C) from the same selection had no flowers pollinated and grew 387 cm. in the same time. More laterals were produced and the tip of the stem showed a luxuriant, vigorous growth as compared to a slender unproductive growth in the other plant. Both were grown under optimum light conditions. Under greatly reduced light the effect of fruit on the growth of this type of plant is not so pronounced because the available light apparently is the controlling factor for stem elongation.

For the prolific "set" type, the plant shown in Figure 12, A, is a good illustration. For this type the time of pollination is important. If a flower has been fertilized several weeks before the plant is subjected to reduced light, the ripening fruit will have some inhibitory effect on the production of pistillate flowers, and not until the fruit is removed or dropped will the final stage shown in Figure 10, B, result. In this case the amount of nitrogen available at certain stages may

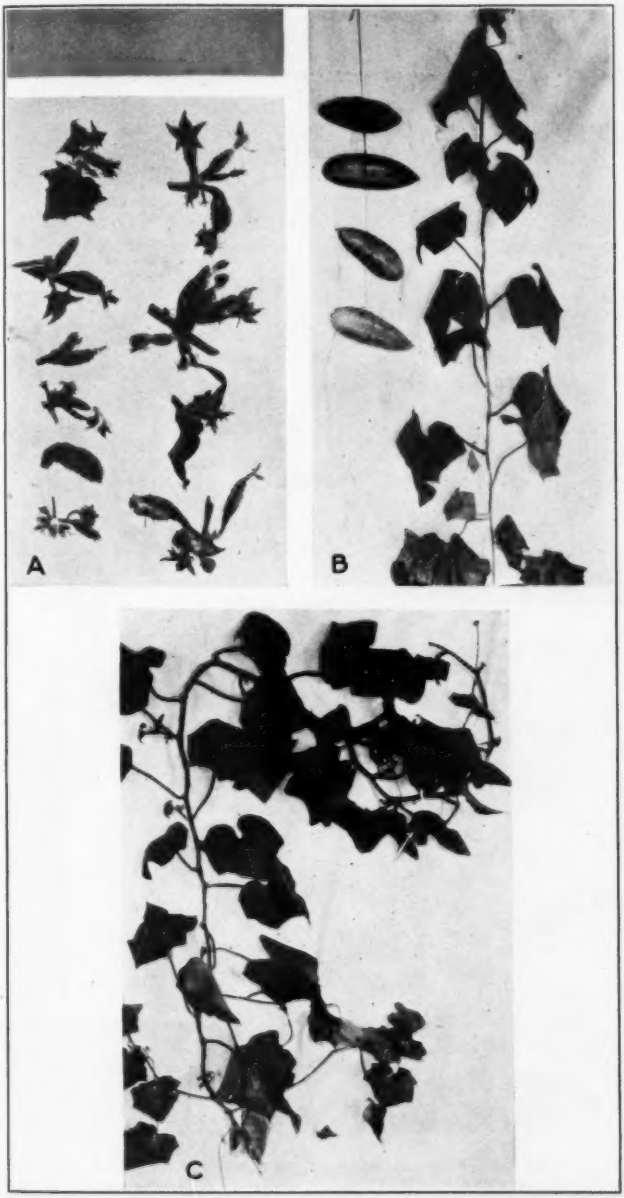

FIG. 11.—A, clusters of pistillate flowers characteristic of the C29-12 type under minimum light conditions; B, plants showing influence of developing fruit on growth; C, a plant from the same selection as B, but having no flowers pollinated

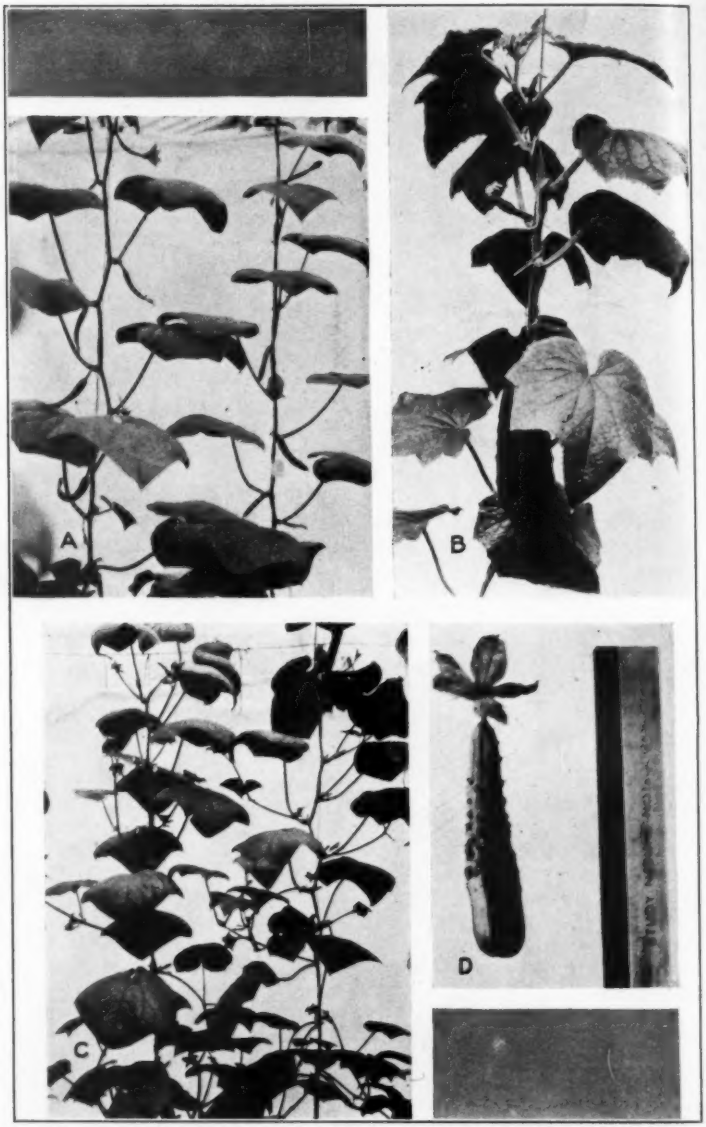

FIG. 12.—A.—C29-12, a pistillate type. The absence of staminate flowers at the nodes is characteristic of plants of this type when grown under partially reduced light. B.—Plant showing effect of heavy nitrogen applications when grown under optimum light conditions. C.—28-4-1, a staminate strain. An occasional pistillate flower is visible on the laterals. D.—A pistillate flower of unusual size at anthesis. Occasionally found on plants during days of reduced sunlight

bring about the extreme fruiting condition. These results are more or less in agreement with Murneek's (*30*) results with tomatoes.

There should be mentioned a few specific effects of nitrogen under a given light condition. In Figure 12, B, is shown a plant grown with optimum nitrogen under favorable light conditions. Parts of this plant are shown in detail in Figure 13, C. This plant produced pistillate flowers at the nodes on the main stem. In Figure 12, C, the type is shown with pistillate flowers on the laterals only. The luxuriant green growth is very conspicuous.

The presence of nitrogen in the soil has a pronounced effect on the size of the flowers. Figure 12, D, shows a flower at anthesis and

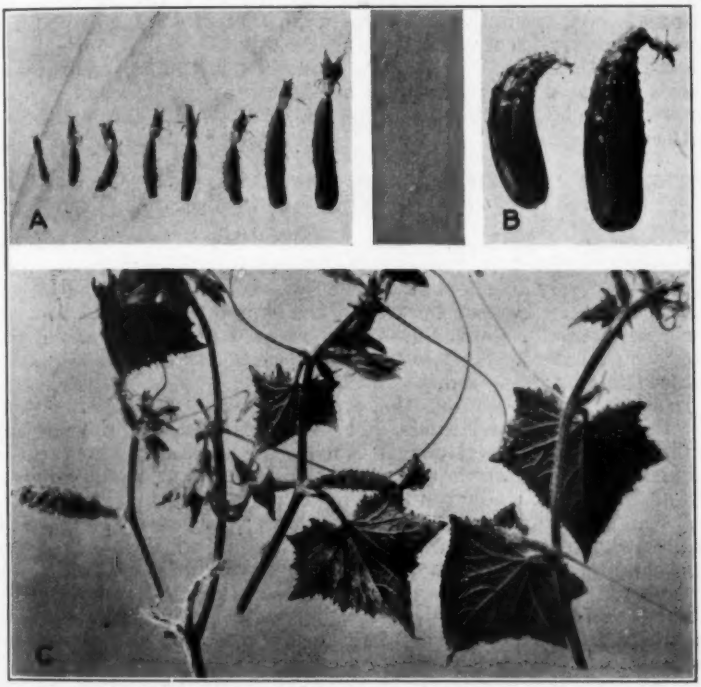

FIG. 13.—A.—Ovaries of flowers 10 hours before anthesis, showing variation in size when plants are grown under reduced light and different conditions of nutrition. B.—Unusually large ovaries of flowers 10 hours before anthesis found on plants grown under reduced light; rather a common occurrence. C.—A closer picture of the young leaves from laterals of plant shown in Figure 12, B

Figure 13, A, shows pistillate flowers 12 hours before anthesis. The difference in size can be attributed to increased amounts of nitrogen. The effect of vigor is shown by some measurements of pistillate flowers taken after all developing fruits had been removed from a vine producing a 30-cm. mature fruit. The ovaries on the first pistillate flowers averaged 5.8 cm. in length at anthesis. Four days later the average at anthesis was 4.8 cm., and in 14 days the average at anthesis was 3 cm.; at which time the first fertilized fruits had attained a length of 24 cm. Thereafter all flowers that did not dry up before anthesis averaged 3 cm. A large number were produced after the maturing

fruits had reached their maximum size but rarely did one reach anthesis, because they turned yellow when the ovary was from 1 to 3 cm. long. Apparently the amount of nitrogen available when all pollinated fruits were removed previous to taking measurements of length of ovaries was in a large measure responsible for the abundant formation of pistillate flower primordia, a condition which prevailed as long as the laterals continued to grow. As the developing fertilized fruits required more nutrients, less was available for the extension of laterals, and the elaboration of pistillate flowers decreased, until finally a large number of them turned yellow before anthesis. Under decreased nitrogen conditions fewer pistillate flower primordia are produced. Although sufficient light is necessary yet the abundant nitrogen above the optimum results in the production of more pistillate flowers, the ovaries of which are considerably larger than normal. In Figure 13, B, ovaries are shown of a type which is common on heavy-set plants during days of minimum sunlight provided the soil is quite fertile. The larger ovary in Figure 12, D, was 7 cm. long just before anthesis.

EFFECT OF ENVIRONMENT ON THE RATIO OF STAMINATE TO PISTILLATE FLOWERS

In selection C29–12, a prolific pistillate flower type, the ratio of staminate to pistillate flowers was changed from 10.8 : 1 during days of comparatively long sunlight to 1.8:1 during days of comparatively short sunlight. Toward the end of the experiment C29–12 was 100 per cent pistillate. This condition is characteristic of plants of this type which have a pistillate flower at practically every node and a reduced number of staminate flowers.

In selection 28–4–1, a selection producing very few pistillate flowers and those only on the laterals, and producing staminate flowers very prolifically at all the nodes, it is possible to change the ratio of staminate to pistillate flowers, but here the type is at the opposite end of the scale. Under optimum light conditions this selection gave 64.0 staminate flowers to 1.0 pistillate flower, as shown in Figure 14, with some plants producing no pistillate flowers, while under reduced light conditions a ratio of 25.5 staminate flowers to 1 pistillate flower is the result with all plants producing some pistillate flowers.

The significance of these facts is that under optimum light conditions C29–12 can be shifted from a normal condition to 100 per cent pistillate, while 28–4–1 can be shifted from practically 100 per cent staminate to the normal type. C29–12 could be shifted to the pistillate type but not to the staminate type, while 28–4–1 could be shifted to the staminate type but not to the pistillate type, all by changes of the environment. Thus, these types are flexible within certain limits, and these limits are undoubtedly controlled by the genetic mechanism of the plant. In C29–12 under optimum light conditions numerous staminate flowers are produced at each node with the pistillate flower. As the light intensity and duration are decreased fewer staminate flowers are produced until in December, under North Temperate Zone conditions, only pistillate flowers occur. The difference here noted in behavior of strains agrees with the results of Correns (*14*) on *Satureia hortensis* (summer savory), in which different strains gave different ratios of perfect to pistillate flowers. In 28–4–1 there is a decrease in staminate flowers, while the

pistillate flowers are only slightly increased. In the case of a commercial variety, the degree of shifting of the ratio would depend on the genetic potentialities of the variety. This fact is very well demonstrated by the Granite State and Belleville varieties. Under reduced light conditions the Granite State has a very narrow sex ratio. Under optimum light conditions the sex ratio is approximately 6 staminate flowers to 1 pistillate flower. In the Belleville strain under reduced light conditions, the sex ratio is normal and becomes wide under optimum light conditions. It is possible to combine certain isolated strains which will produce sex ratios within certain desired limits.

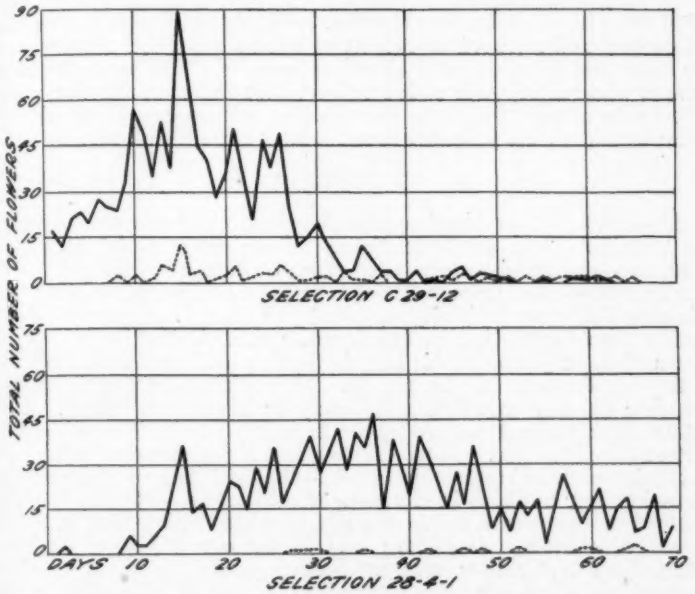

FIG. 14.—Comparison of selections C29-12 and 28-4-1 for distribution of pistillate and staminate flowers when grown under comparable conditions from September to November. Dotted lines show daily production of pistillate flowers; solid lines show daily production of staminate flowers. Compare with plants in Figure 1, grown during days of maximum sunlight

Cytological data of a preliminary nature using Bellings aceto-carmine method have not disclosed any departures from the normal seven haploid chromosomes established by Kozhukhov (*25*) and later by Heimlich (*22*).

Preliminary data on the genetics of greenhouse cucumbers indicate that pistillate and staminate flowers result from factor genes on the chromosomes rather than from any difference in the number or shape of the chromosomes. These conclusions are in agreement with Emerson's (*17*) hypothesis that sex in plants is determined by genetic factors just as any other characters are determined by genes on the chromosomes.

Data on staminate and pistillate flowers of an F_2 population show plants having a large number of staminate flowers and very few

pistillate flowers with all intergrading variations to the type that has abundant pistillate flowers and very few staminate flowers under a given set of environmental conditions. From the data the conclusion may be drawn that or any given selection, optimum light conditions (abundant carbohydrates) favor the abundant production of staminate flowers even where nitrogen in quantity considerably above the optimum is applied to the soil. It may also be concluded that reduced light conditions favor the production of pistillate flowers with a decided decrease in staminate flowers regardless of an appreciable fluctuation of the nitrogen content of the soil. This suggests a correlation between flower formation and the amount of available carbohydrates and nitrogen which Kraus and Kraybill (*26*) call the nitrogen-carbon ratio. Whether it is due to the ratio of carbon and nitrogen or carbon and some other soil element must be determined by a careful chemical analysis. The reactions of the plants when nitrogen is added suggest that flower production within the limits of gene flexibility is correlated with some carbon-nitrogen relationship which probably is not a fixed ratio for all plants.

DISCUSSION

Manoilov (*27*) combined certain chemicals which reacted differently to staminate and pistillate tissue. Tests were made with these reagents, and these tests showed plants which were almost 100 per cent pistillate, as selection C29–12, to have a different reaction from plants which were decidedly staminate, as 28–4–1. Leaves of plants which were segregating in the F_2 generation showed decided differences when subjected to this test, indicating a difference in the metabolic constituents of the plants. This difference was noticeable whether the day was dull and cloudy or bright and sunny. Tests made on young and mature leaves from the same plant showed no detectable difference. These observations are in agreement with certain microchemical tests which showed that pistillate plants were deficient in sugar but high in nitrogenous substances. Staminate plants showed more sugar in the leaves but were also rather high in nitrogenous substances. Alsterberg and Håkansson (*3*) have criticized Manoilov's test rather severely because positive results were not secured on all plants. Undoubtedly the test is influenced by the homo- or hetero-zygosity of the sex condition, the former probably giving a better test than the latter. It was found in tests on cucumbers that extreme care is necessary in applying the test.

The general conclusion from the data lends support to the hypothesis that sex is determined by some genetic-factor mechanism which controls the metabolism in the plants rather than the actual character of sex expression, and that the ratio between the two types of flowers is the result of this nutrient condition. Each plant may have a definite nutrient ratio which is thrown one way or the other by light and by soil nutrients. Kraus and Kraybill (*26*) suggest a nitrogen-carbon ratio as controlling the reproductive stage in tomatoes. If this ratio is in a normal pistillate plant containing the recessive factor for flower location as in selection C29–12, a decrease in light will throw it toward the extreme pistillate type. Where the ratio is accompanied by the dominant allelomorph producing pistillate flowers only on the laterals, as in 28–4–1, a decrease in light will likewise cause the plant to produce more pistillate flowers, but here,

because of the inhibition of pistillate flowers on the nodes of the main stem, the ratio remains rather wide.

The presence or absence of fruit may influence this ratio somewhat, but in experiments in which all the pistillate flowers were removed the plants immediately proceeded to repeat the previous cycle, in keeping with their genetic potentialities under a given environment. The effect of the presence of fruit and other conditions of environment may be seen, but their influence is confined within certain limits established by the genetic factor mechanism of the plant.

Just how this relationship interacts is a question that a very careful chemical study may answer. The retarded growth of the C29–12 type is not due to the heavy production of pistillate flowers, for there are types which are free growing and produce pistillate flowers on practically all the nodes. Selection 28–4–1 is a free-growing type without the pistillate flowers on the nodes of the main stem. From breeding data it would seem as though some plants are endowed with certain potentialities which decide their ratio of carbohydrates to soil nutrients and that the production of pistillate and staminate flowers is the result of this ratio. Selection C29–12 would then produce all pistillate flowers when the ratio stood, let us say, 1 carbon unit to 5 soil-nutrient units, while in 28–4–1 the ratio would have to be 1 carbon unit to 10 soil-nutrient units to produce only pistillate flowers; a condition which has never been observed in these experiments. Under a 1 : 10 ratio, selection 28–4–1 might be induced to produce pistillate flowers on the nodes of the main stem. Even were this condition attained, it would not invalidate the genetic hypothesis discussed above, for there would still be the previously noted difference between the two selections, which can only be explained by some basic determining mechanism. The inhibited type of growth, as in C29–12, seems to possess a tendency to utilize the sugars for protein metabolism, while the 28–4–1 type has a tendency to accumulate sugars. It is possible that the genetic factor influences the quality of the sugar in the different types of plants, so that the reduction of nitrates might result in differently constituted proteins which would have their respective resultants in the appearance of the plants and their behavior for flower formation.

The general conclusion would support the hypothesis suggested by Emerson (*17*) that sex is determined by some factor or gene on one or more pairs of chromosomes, which is more or less flexible to allow for the effect of environmental changes. It further suggests that the sex ratio is the result of a chemical balance in the plant which probably is the medium through which the factors show their influence. Whether the initial cell produces a staminate or a pistillate flower probably depends on the inter- and intra-cell nutrients. In the 28–4–1 type where a factor inhibits the production of pistillate flowers at certain nodes, these cell nutrients will result in either staminate flowers or stem and leaf tissue, depending on environmental conditions. Where the inhibitory factor is lacking, pistillate flowers will be formed accompanied by staminate flowers or other pistillate flowers, depending on environmental conditions. If conditions are not conducive to the formation of a single pistillate flower the node may remain barren.

Some light is thrown on this phase of the subject by observations on plants low in nitrogen or nutrients. Suppose the plant has pro-

duced a large number of staminate buds. If a supply of nitrogen is suddenly available, the plant is forced to slough off these staminate buds and produce either pistillate flowers or tissue in the form of laterals, depending on the type of plant under observation.

Thus the data seem to point to a chromosome factor or factors for sex determination, even though environment, by changing the nutrient balance, can change the sex ratio within certain limits. These limits are either one or the other flower type or vegetative growth. It can not be said that environment causes a change from a staminate to a pistillate flower condition, but rather that it causes a change from one or the other types of flowers to leaf and stem tissue. Environment, therefore, does not determine the sex; it merely produces conditions which make possible the expression of potentialities in the plant.

SUMMARY

Environmental factors as they affect the production of pistillate and staminate flowers on cucumber plants were studied.

An abundance of light tends to increase the number of staminate flowers, within certain limits.

The reduction of light materially increases the number of pistillate flowers and decreases the number of staminate flowers.

The use of electric light at an intensity of 100 foot-candles has a marked effect in increasing the number of staminate flowers during days of minimum sunlight.

The presence of fruit exerts an inhibitory effect on the development of the plant as well as on the production of pistillate flowers.

Certain selections having a heavy production of pistillate flowers at the nodes of the main stem show an absence of staminate flowers. These selections under reduced light conditions produce false parthenocarpic fruits when pollination is not accomplished, and even when it is accomplished many of the fruits are not fertilized.

The heavy production of pistillate flowers on a pistillate type is associated with fasciation which causes the plants to become more or less dwarfed because of the clusters of pistillate flowers which take the place of the growing tips.

Preliminary data on the production and location of pistillate flowers on cucumber plants indicate that these characters are controlled by some chromosome mechanism.

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THE ANTIRACHITIC PROPERTIES OF COD-LIVER MEALS[1]

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INTRODUCTION

In the production of cod-liver oil from the livers of the codfish (*Gadus callarias*) there remains a residue which in recent years has been dried and sold in the open market under the name "cod-liver meal." The nutritional value of this by-product of the cod-liver oil industry is creating a great deal of interest among poultrymen, poultry feed manufacturers, and animal feeders. It has been claimed that this liver residue, aside from the quality of its proteins, carries certain vitamin properties, principally those of an antirachitic nature.

It has been shown in the station laboratory, as well as by investigators [2] elsewhere, that cod-liver oils may vary greatly in their vitamin A and D content. This variation in vitamin potency is directly influenced by the amount of these factors present in the cod livers from which the oil is extracted. The method employed in the extraction of the oil may also exert a secondary effect on its vitamin content. Likewise, the residue remaining after the partial extraction of the fats would be expected to vary in vitamin properties, depending upon the original vitamin content of the livers, the amount of oil remaining in the residue, the method employed in expelling the oil, and the procedure used in drying the liver residue. Accordingly it appeared logical to inquire into the fat-soluble vitamin content of this product. The results obtained on the antirachitic properties of cod-liver meals form the basis for this report.

EXPERIMENTS WITH CHICKS

The first experiments on the antirachitic properties of liver residues were conducted with chicks. For this purpose 156 day-old White Leghorn chicks hatched from eggs laid by hens of the same nutritional history were divided into 6 lots of 26 each. Each lot was confined in an indoor pen 3 by 6 feet. The only sunlight available was that which filtered through closed, muslin-covered windows.

Lot 1 received a ground mash of yellow corn 57 parts, wheat middlings 20, dried buttermilk 15, meat meal 5, calcium carbonate 2, and sodium chloride 1. Lots 2, 3, and 4 received a similar ration in which the 5 parts of meat meal were replaced by an equal quantity of a different cod-liver meal in each lot. The ration of lot 5 was the same as that of lot 1, with the addition of 2 parts cod-liver oil. Lot 6 received a ration in which liver meal No. 3 served as the main source of protein as well as the antirachitic factor. It had the following composition: Yellow corn, 65 parts; wheat middlings, 20;

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[2] Heuser, G. F., and Norris, L. C. Rickets in chicks. I. Variations in the antirachitic potency of different brands of cod-liver oil. II. Variations in the antirachitic potency of different grades of cod-liver oil. Poultry Sci. 6: 9-17, 94-98, illus. 1926-27.

cod-liver meal (No. 3), 12; calcium carbonate, 2; and sodium chloride, 1. All lots were cared for alike. The chicks were weighed individually every week. Pine shavings, changed weekly, were used as litter. Water was given as a drink.

The cod-liver meals used in the experiments were obtained from three different manufacturers and represented the dried liver residue remaining after the removal of the oil from fresh livers by the steam process. Meals 1 and 3 were of American manufacture; meal 2 was of Norwegian origin. Although no direct information as to the method and temperature employed in the manufacture of meals 1 and 2 was obtainable, it was apparent from the charred appearance of meal 2 that it had been subjected to a rather high temperature. Meal 1 in this respect presented a somewhat better appearance, but was darker in color than meal 3, which, according to the manufacturer, had been dried in a special vacuum drier at 70° F.

TABLE 1.—*The effect of feeding different cod-liver meals or cod-liver oil on the growth of chicks and the ash content of their tibiae*

Age of chicks (weeks)	Lot 1, fed basal ration (control)			Lot 2, fed ration containing 5 per cent cod-liver meal 1			Lot 3, fed ration containing 5 per cent cod-liver meal 2		
	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness
	Grams			*Grams*			*Grams*		
1	55.9	26	0	52.7	26	0	52.2	26	0
2	75.2	26	0	69.6	26	0	68.1	26	0
3	104.4	26	17	108.3	26	2	100.9	26	10
4	132.5	26	26	146.6	26	7	131.1	26	18
5	160.5	25	25	201.1	26	16	158.4	26	26
6	189.8	25	25	238.4	26	18	175.6	26	26
Average per cent of ash in tibiae (fat free)	32.23±.22			39.19±.69			32.32±.37		

Age of chicks (weeks)	Lot 4, fed ration containing 5 per cent cod-liver meal 3			Lot 5, fed ration containing 2 per cent cod-liver oil			Lot 6, fed ration containing 12 per cent cod-liver meal 3		
	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness
	Grams			*Grams*			*Grams*		
1	49.6	26	0	50.3	26	0	53.6	26	0
2	67.7	26	0	74.8	26	0	69.8	26	0
3	109.0	25	0	113.1	26	0	100.3	26	0
4	163.0	25	0	160.9	26	0	132.3	26	0
5	221.8	25	0	218.7	26	0	155.9	26	0
6	268.1	25	1	279.6	26	0	202.4	26	0
Average per cent of ash in tibiae (fat free)	44.14±.58			47.61±.35			47.58±.21		

The chicks in the first four lots were continued on the experiment for six weeks, when 12 representative birds from each of lots 1, 2, 3, and 4 were killed for bone analysis. At the same time six birds were taken from each of lots 5 and 6. The remaining 20 birds in each of the two last-mentioned lots were continued on the experiment to the eleventh week. For bone analysis the tibiae were removed, freed from adhering tissue, dried, crushed, then extracted with hot alcohol and ether, and subsequently ashed in an electric muffle furnace. The data are recorded in Table 1. The percentage of ash is expressed on the fat-free basis.

It is evident from the results secured (Table 1) that the three cod-liver meals varied markedly in their antirachitic properties. Meal 2, fed lot 3, exerted no antirachitic effect, as is attested by the number of cases of leg weakness and by the ash content of the tibiae. Meal 1 proved to be better from a calcification standpoint than meal 2—accounting for an approximate 7 per cent increase in ash content over the basal ration (lot 1) and liver meal 2 (lot 3). Although meal 3 proved the most efficient of the three liver residues—making for an increased ash content of approximately 12 per cent over the control group (lot 1) at a 5 per cent level of intake—it did not prove antirachitically equivalent to 2 per cent cod-liver oil (lot 5). Increasing the intake of meal 3 to 12 per cent (lot 6) resulted in an apparently normal ash content of the tibiae.

The growth of the chicks in all lots except 6 paralleled the vitamin-D intake. Meal 3 again proved superior to the other two meals, but not quite equivalent to 2 per cent cod-liver oil. The slower growth of lot 6 may in part be accounted for by the lowered protein intake. The 20 birds that remained in lots 5 and 6 at the sixth week and continued on the same rations weighed 718.7 and 408.5 grams, respectively, at the eleventh week. No cases of leg weakness were observed in either lot, and from all appearances the birds were all normal except for the retarded growth of lot 6. There were evidences of mild digestive disorders when the meal was fed at a 12 per cent level.

TABLE 2.—*Composition of the three cod-liver meals*

Sample	Moisture	Ash	Ether extract	Protein (N×6.25)
	Per cent	*Per cent*	*Per cent*	*Per cent*
Cod-liver meal 1	8.70	4.10	30.70	43.56
Cod-liver meal 2	5.88	3.25	23.21	54.18
Cod-liver meal 3	6.21	3.54	44.19	39.06

A chemical analysis of the three liver meals revealed a marked variation in their protein and residual fat content (Table 2). Meal 3 contained approximately twice as much ether-extractable material as meal 2, while meal 1 occupied an intermediate position. These facts suggested that the amount of protection which these meals were able to exert against leg weakness (experiment 1) was proportional to the residual fat content. To test out this hypothesis, 80-day-old White Leghorn chicks of the same nutritional history were divided into four lots of 20 each and fed a ration similar to that used in experiment 1, except that the different meals were so incorporated as to supply approximately 3 per cent of ether extract. To make

the rations fairly comparable as to total protein, the dried buttermilk was reduced accordingly. The rations had the following composition:

	Lot 1	Lot 2	Lot 3	Lot 4		Lot 1	Lot 2	Lot 3	Lot 4
Yellow corn	55	57	59	55	Cod-liver meal		10	13	7
Wheat middlings	20	20	20	20	Calcium carbonate	2	2	2	2
Dried buttermilk	15	10	5	15	Sodium chloride	1	1	1	1
Meat meal	5				Cod-liver oil	2			

Lot 2 received meal 1; lot 3, meal 2; and lot 4, meal 3.

All four lots were housed in separate 3 by 6 foot pens, located in the poultry building. Direct sunlight was not accessible. Planer shavings were used as litter. Fresh water was supplied daily.

TABLE 3.—*Effect on chicks produced by feeding cod-liver oil or by feeding different cod-liver meals on the same ether-extract basis*

Age of chicks (weeks)	Lot 1, fed ration containing 2 per cent cod-liver oil			Lot 2, fed ration containing 10 per cent cod-liver meal 1			Lot 3, fed ration containing 13 per cent cod-liver meal 2			Lot 4, fed ration containing 7 per cent cod-liver meal 3		
	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness	Weight	Number surviving	Number with leg weakness
	Grams			*Grams*			*Grams*			*Grams*		
1	51.9	20	0	50.9	20	0	50.0	20	0	53.3	20	0
2	70.9	20	0	76.9	20	0	63.5	20	0	79.2	20	0
3	119.2	20	0	110.7	20	0	87.8	20	0	114.2	20	0
4	169.5	20	0	153.2	20	0	107.1	19	6	152.3	20	0
5	222.5	20	0	191.7	20	0	121.9	18	12	194.5	19	0
6	292.3	19	0	256.3	19	4	147.3	16	16	256.0	19	0
Average per cent of ash in tibiae (fat free)	48.97±.32			41.47±.64			37.07±.27			47.08±.42		

The chicks, as in the preceding test, were continued on the experiment for six weeks, when 10 representative birds from each lot were killed for bone analysis as before. The results are tabulated in Table 3. Meal 3, although fed at the lowest level, again proved the best antirachitically, followed in order by meals 1 and 2. The ash content of the tibiae from the groups fed cod-liver meal, lots 2, 3, and 4, was approximately from 2 to 5 per cent higher than that for the same lots in experiment 1. This somewhat better calcification may be explained on the basis of increased vitamin-D intake. The writers are inclined to believe, however, that the higher ash content, particularly in the case of lot 3, which received meal 2, was influenced by the increased phosphorus intake in establishing a more favorable calcium-phosphorus relationship for normal bone formation. Later work with rats substantiates the contention that the better calcification noted with meal 2 in experiment 2 was not due to increased vitamin-D intake. In general, the results of experiment 2 are in accord with those of the first experiment. They also show that the antirachitic properties of these meals bear no direct relation to their fat content.

EXPERIMENTS WITH RATS

To obtain further knowledge of the antirachitic value of the different cod-liver meals, the writers used the rat as the experimental animal. The rat represents a rather rapidly growing animal, which can be kept under better nutritional control than the chick, and consequently affords better analytical results.

Bethke, Steenbock, and Nelson [3] pointed out some time ago that with the rat on low calcium rations the amount of cod-liver oil necessary to furnish the antirachitic factor in sufficient amounts varied inversely with the calcium content of the diet. The same conclusion was reached in the case of rats on relatively high phosphorus diets. Other unpublished work with this animal has convinced the writers that the ratio of calcium to phosphorus in the diet bears a direct relation to the antirachitic requirements of this species for normal calcification. Since cod-liver meal contains a relatively high percentage of phosphorus, it is obvious that the addition of this product to a rickets-producing ration would affect the calcium-phosphorus relation and make conditions more favorable for calcification. In order not to complicate the results by a change or adjustment in the calcium-phosphorus relation, the writers decided to use the extracts of these meals.

It was provisionally assumed that the major portion of the antirachitic properties of these liver meals was present in the residual fat, and therefore would be removed by a fat solvent like ether, as suggested by the work of Shipley, Kinney, and McCollum.[4] Although it is not safe to conclude from the work of these investigators that all of the antirachitic factor is extracted by ether, the vitamin-D content of the various extracts at least should be comparable if they are prepared under the same conditions.

The experiments with rats were carried out by feeding the cod-liver meal extracts at levels of 0.5 per cent in a rickets-producing ration. The ration employed was the one reported by Steenbock and his associates,[5] which consisted of yellow corn 76 parts, wheat gluten 20, calcium carbonate 3, and sodium chloride 1. The extracts were prepared by extracting the meals for 24 hours with ether in a large Soxhlet extractor. The excess ether was removed from the extract by distillation under reduced pressure. The resulting oily residue was then added directly to the ration.

The rats used were raised in the station laboratory under standardized conditions. They were taken at the age of 25 to 28 days, weighing from 50 to 64 grams. In all, 5 litters of 6 rats each were divided into 6 lots of 5 each. One lot was killed at the beginning of the experiment and the femurs removed for ash analysis. Another lot received the basal rachitic ration and served as a control. Three more lots were fed the rachitic basal mixture fortified with 0.5 per cent of the

[3] BETHKE, R. M., STEENBOCK, H., and NELSON, M. T. FAT-SOLUBLE VITAMINS. XV. CALCIUM AND PHOSPHORUS RELATIONS TO GROWTH AND COMPOSITION OF BLOOD AND BONE WITH VARYING VITAMIN INTAKE. Jour. Biol. Chem. 58: 71–103, illus. 1923.

[4] SHIPLEY, P. G., KINNEY, E. M., and MCCOLLUM, E. V. STUDIES ON EXPERIMENTAL RICKETS. XXIV. THE EFFECT OF CERTAIN EXTRACTS OF PLANT TISSUES ON FLORID RICKETS. Jour. Biol. Chem. 59: 165–175. 1924.

[5] STEENBOCK, H., HART, E. B., ELVEHJEM, C. A., and KLETZIEN, S. W. F. DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION. VI. THE ANTIRACHITIC PROPERTIES OF HAYS AS RELATED TO CLIMATIC CONDITIONS WITH SOME OBSERVATIONS ON THE EFFECT OF IRRADIATION WITH ULTRA-VIOLET LIGHT. Jour. Biol. Chem. 66: 425–440, illus. 1925.

ether extract of the different liver meals; the remaining lot received the basal mixture supplemented with 0.5 per cent crude medicinal cod-liver oil. The five groups of rats were fed for a period of four weeks, when they were chloroformed and the femurs removed for ash analysis. For this purpose the bones were freed from tissue, dried at 50° C. for 24 hours, crushed, then extracted with hot alcohol and ether for 36 hours, dried, and ashed in a muffle furnace. The results are recorded in Table 4.

TABLE 4.—*The calcifying properties of the ether extract of different cod-liver meals and of cod-liver oil fed to rats as an addition to a rickets-producing ration*

Lot No.[a]	Addition to ration	Average initial weight	Average final weight	Average ash in femurs
		Grams	*Grams*	*Per cent*
1349	None (control lot, killed at begining of experiment)	57	----------	39.32±.46
1354	None (rachitic control)	58	89	27.03±.85
1350	0.5 per cent ether extract of cod-liver meal 1	58	89	29.50±.91
1351	0.5 per cent ether extract of cod-liver meal 2	56	90	26.78±.86
1352	0.5 per cent ether extract of cod-liver meal 3	58	87	38.19±.83
1353	0.5 per cent cod-liver oil	57	80	45.85±.39

[a] Each lot contained 5 rats.

It is seen in Table 4 that over the four-week period the ash content of the femurs from rats in lot 1354, fed the unmodified rickets-producing ration, markedly decreased as compared to the average of 39.32 per cent for the control lot killed at the beginning of the experiment. The addition of 0.5 per cent of the ether extract of cod-liver meal 2 to the basal ration exerted no favorable effect on calcification. This observation substantiates the results obtained with chicks. The 0.5 per cent of the extract from meal 1 gave evidence of affording a slight antirachitic effect, but did not prove nearly as efficient as extract from meal 3. The latter, however, did not furnish sufficient vitamin D at a 0.5 per cent level to produce a normal ash content, as did 0.5 per cent cod-liver oil. The results secured with rats are in general agreement with those obtained with chicks.

To gain further knowledge of the calcifying properties of the extracts from cod-liver meals in comparison with the cod-liver oil previously used, the line-test method as described by McCollum and his coworkers [6] was employed. The rats used for these tests were animals of the writers' own breeding, started when from 25 to 28 days of age and weighing approximately 60 grams on the Steenbock rickets-producing ration previously described. After 24 days on this ration, when moderate rickets had developed in all rats, the diets were supplemented for 10 days with varying amounts of cod-liver oil and the oily extract of the meals thoroughly incorporated in the ration. At the end of this time the rats were killed with ether and the radii and ulnas removed and examined for calcium deposition by staining with silver nitrate.

[6] MCCOLLUM, E. V., SIMMONDS, N., SHIPLEY, P. G., and PARK, E. A. STUDIES ON EXPERIMENTAL RICKETS. XVI. A DELICATE BIOLOGICAL TEST FOR CALCIUM-DEPOSITING SUBSTANCES. Jour. Biol. Chem. 51: 41–49, illus. 1922.

TABLE 5.—*Calcium deposition in rachitic rats after the rickets-producing ration was supplemented by the ether-extract of different cod-liver meals or by cod-liver oil*

Supplement to rickets-producing ration	Rat No.	Weight	Average daily consumption	Calcium deposition *
		Grams	*Grams*	
Ether extract of cod-liver meal 1:				
0.40 gram	4434	73–87	6.5	−
	4439	80–80	6.5	−
0.60 gram	4435	75–89	7.0	−
	4441	71–79	7.0	−
1.00 gram	4437	81–92	7.0	−
	4443	80–88	7.5	+
Ether extract of cod-liver meal 2:				
0.60 gram	4438	93–100	8.0	−
	4446	83–86	7.0	−
1.00 gram	4442	69–75	7.5	−
	4447	74–77	5.5	−
2.00 grams	4444	79–90	8.0	−
	4449	82–83	6.5	−
ther extract of cod-liver meal 3:				
0.40 gram	4453	91–102	8.0	−
	4458	88–90	7.0	−
0.60 gram	4454	74–84	6.0	−
	4459	92–96	8.0	++
1.00 gram	4455	81–92	7.0	+
	4460	83–88	7.5	++
Cod-liver oil:				
0.10 gram	4448	80–81	7.0	++
	4456	85–99	8.5	++
0.20 gram	4450	74–85	7.5	+++
	4452	74–80	6.0	+++

* −=No calcium deposition; +=evidence of calcium deposition; ++=narrow line of calcium; +++=wide line of calcium.

The results in part, as recorded in Table 5, show that the cod-liver oil used in all these experiments was at least 6 times as potent antirachitically as the extract from meal 3 and more than 10 times as active as the extract from meal 1. The oily extract of meal 2 apparently possessed very few if any antirachitic properties, which is in agreement with the results obtained in feeding the meal to growing chicks.

SUMMARY AND CONCLUSIONS

The results of experiments with chicks and rats show conclusively that the dried residues remaining after the extraction of oil from fresh cod livers vary markedly in their antirachitic properties. The antirachitic variation was not proportional to the residual fat content of the livers. Nor did the ether-extractable fraction prove nearly as potent as ordinary cod-liver oil. Accordingly, it would seem unwise to use the liver meal as an antirachitic substitute for a good grade of cod-liver oil in either poultry or livestock production.

Whether cod-liver meals may possess other merits aside from their questionable fat-soluble vitamin content remains to be determined.

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